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NEW ASPECTS OF RELATIVITY

GEOMETRICAL TREATMENT OF THE VOIGT AND PAGE TRANSFORMATIONS*

By ARCHIBALD HENDERSON

3 TEXT FIGURES

(I)

On March 26 of the present year, the most precious piece of glass in the world, estimated in money value at \$6,000,000.00, left Corning, New York, on its 3,300-mile trans-continental journey to Pasadena, California. This is the 200-inch telescope-eye designed for erection on Mount Palomar, north of San Diego, California. The completion of this monster eye-piece, a twelve-years task first and last, is one of the supreme triumphs of scientific, inventive, and technical skill of modern times.

The new telescope will be, it is estimated, about 360,000 times as effective as the human eye. It will plumb the inter-stellar spaces three times as far and expose a volume of space twenty-five times as vast as the 100-inch reflector on Mount Wilson will now do. But whether it will confirm the validity of the theory of relativity or determine whether the universe is at present expanding, is problematical. Queried on the former issue, Einstein replied: "Not the eye but the spirit furnishes the proof of theories."

During the past three decades, the theory of relativity has revolution-

* This paper, in substance, was delivered before the Southeastern Section, Mathematical Association of America, University of South Carolina, Columbia, April 17, 1936.

ized the thinking of mathematicians, physicists, astrophysicists, and astronomers. It has given an incalculable impetus to research, in especial along the lines of higher forms of space, tensor calculus, differential geometry, non-Euclidean geometry, light, optics, and electromagnetism. Einstein himself, Eddington, Weyl, and others have labored to generalize the field-theory conception to combine electromagnetism and universal gravitation in a logical and coherent unity. Not only is the Einstein theory of relativity being intensively studied: new theories of relativity, suggested by Einstein's pioneering and now classical contributions, are being advanced, notably by Sulaiman, Milne, and Page. These newer theories, like the older, are characterized by formal speculation and imaginative intuition; and in the last analysis assume the "uniformity of the secrets of natural law." It is no longer desirable, as Einstein has recently pointed out, for the physicist to abdicate in favor of the philosopher, in the matter of critical contemplation of the theoretical foundations of physics. "It cannot be right at a time when the very foundations of physics itself have become problematic as they are now."¹

(II)

The foundation principles of Einstein's restricted relativity theory are: the famous Voigt transformation,² incorrectly ascribed to Lorentz; the invariability of a quantity known as the Riemannian space-time interval, a composite of both space and time; and the principle of the constancy of light-velocity in empty space. In his researches in elastic light-theory, Voigt in 1887 derived the famous formula, for the purpose of introducing the concept of "local time" in a mobile system and thereby establishing the validity of the wave-equation

$$\Delta\phi - \frac{1}{c^2} \frac{\partial^2\phi}{\partial t^2} = 0$$

in the mobile system. In several writings of a fundamental character, dating from 1892, Lorentz effectively established, by means of this same transformation independently arrived at, the physical equivalence

¹ Albert Einstein: "Physics and Reality." *Journal of the Franklin Institute*, vol. 221, no. 3, March, 1936.

² W. Voigt: "Ueber das Doppler'sche Princip." *Gottinger Nachrichten*, 1887, p. 41.

of the moving and the stationary systems, with rectilinear motion and fixed relative velocity.³ Lorentz however was bound to the old ideas, since he used a privileged system, namely one "fixed in the ether." The revolution in ideas regarding time and space, introduced by Einstein in 1905 in his classic paper "On the electro-dynamics of moving bodies," gave a completely new interpretation of the Voigt transformation.⁴ Aiming at a theory of correlativity, which has since been known under the somewhat inexpressive name of relativity, Einstein denied the existence of any privileged system in space and interpreted the two systems involved as having perfect reciprocity. Einstein treated the Voigt equations as a linear transformation in pseudo-Euclidean space-time, adjusting the time and space co-ordinates of the two systems in order to establish the invariance of light-velocity.

In 1906 Poincaré, in a treatment of the dynamics of the electron and the subject of universal gravitation, interpreted the Voigt equations as a transformation representing a rotation in space through an imaginary angle.⁵ Minkowski in 1907 followed Poincaré in giving a like interpretation of the Voigt transformation,⁶ but in later papers he put forward his ingenious conception of world-lines and the light-cone, usually simplified to the case of the rectangular hyperbola $c^2t^2 - x^2 = 1$, the Voigt equations constituting the transformation from this form to

³ H. A. Lorentz: "La théorie électromagnétique de Maxwell et son application aux corps mouvants." *Arch. Néerl.* **25** (1892), p. 363; "Versuch einer Theorie der elektrischen und magnetischen Erscheinungen in bewegten Körpern" (Leyden, 1895); "Electromagnetic phenomena in a system moving with any velocity smaller than that of light." *Proc. Amsterdam Acad.* VI (1904), p. 809. In his "The Theory of Electrons," being lectures delivered at Columbia University, New York, in 1906 (Leipzig, Teubner, 1909), Lorentz (p. 198), after stating that Voigt's paper had hitherto escaped his attention, specifically acknowledges: "The idea of the transformation might therefore have been borrowed from Voigt, and the proof that it does not alter the form of the equations for the free ether is contained in his paper."

⁴ A. Einstein: "Zur Elektrodynamik bewegter Körper." *Annalen der Physik* **17** (1905), p. 891.

⁵ H. Poincaré: "Sur la dynamique de l'électron." *Rend. del Circolo mat. di Palermo*, vol. xxi, 1906, p. 129.

⁶ H. Minkowski: "Grundgleichungen für die elektromagnetischen Vorgänge in bewegten Körper." *Gött. Nach.*, 1908, p. 53; "Das Relativitätsprinzip." Vortrag gehalten in der math. Gesellsch. zu Göttingen, Nov. 5, 1907, published in *Jahresber. d. Deutsch. Math. Ver.* **24** (1915), p. 372, and in *Ann. d. Physik* **47** (1915), p. 927.

$c^2t^2 - x^2 = 1$, being the same curve referred to a (non-perpendicular) pair of conjugate diameters.⁷

(III)

The restricted relativity theory has received only slight treatment, comparatively speaking, by means of hyperbolic functions of a real variable.⁸ The most thoroughgoing treatment, employing hyperbolic functions, is found in Fontené's brief work on restricted relativity;⁹ but the derivation of the Voigt transformation given by Fontené bears no resemblance to the treatment which follows; nor does Fontené derive geometrically the two invariants, one absolute, the other relative, associated with the Voigt transformation. The following treatment is presented as a complete geometrical representation, by means of hyperbolic functions, of the quantities employed in the Voigt transformation, together with the two associated invariants.

Two systems, S, S' with axes of abscissas in coincidence, move with uniform relative velocity v along their common axis. (See Fig. 1.) Time in each system is measured from the instant ($t = 0, t^1 = 0$) when O and O^1 are in coincidence. When O^1 leaves O a light-ray is sent from this common origin along the direction of motion; and according to the observer at O the observer on the second system arrives at O^1 when the light-ray reaches C . An event occurs at the point $X(x, t)$ in the system S .

There are four problems to be solved:

- 1) How far will the observer at O^1 estimate himself to be from X ?
- 2) How long after the beginning of the journey, namely from O , will the observer at O^1 estimate that the event at X occurred?
- 3) What is the relative invariant of the required transformation?
- 4) What is the absolute invariant?

Representing the light-velocity by c , let us choose $c, v, \sqrt{c^2 - v^2}$ as proportional to $\cosh u, \sinh u, 1$. Then we may set

$$\frac{v}{c} = \tanh u \text{ and } \sqrt{1 - v^2/c^2} = \operatorname{sech} u,$$

since $\operatorname{sech}^2 u = 1 - \tanh^2 u$.

⁷ H. Minkowski: "Grundgleichungen für die elektromagnetischen Vorgänge in bewegten Körpern." *Math. Ann.*, 68 (1910), p. 472; "Raum und Zeit," lecture delivered during the meeting of the "Naturforscherversammlung" at Cologne, Sept. 21, 1908 and printed in *Physik. Zeitschrift* 10 (1909), p. 104.

⁸ W. Pauli, Jr.: *Relativitätstheorie*. (Leipzig, Teubner, 1921), 567, 623; *Bibl.* 3, 58, 73.

⁹ G. Fontené: *La relativité restreinte*. (Paris, Vuibert, 1922).

secting the verticals through M, O¹, X, C, F, A₁, B₁ in the following points: B, W, Z, J, H, G₁; D; Y, G, D₁, E₁; C₁, F₁.

Now OC = OD = ct; OO¹ = vt; OX = OR = x; O¹X = x - vt. Let OQ meet unit circle, centre O, in E; and drop perpendicular, EA, from E to line OO¹. Now we have

$$O^1Q:MR:AE::OQ:OR:OE::OO^1:OM:OA$$

Hence

$$\frac{OA}{OE} \equiv \frac{OA}{1} = \frac{OO^1}{OQ} = \frac{vt}{ct} \equiv \frac{v}{c},$$

Hence OA = tanhu. Then AE = sechu, since sec²hu = 1 - tan²hu. Draw ES parallel to AO and construct the inverse point, N, of S with respect to the unit circle. Then

$$ON \equiv \frac{1}{OS} = \frac{1}{AE} = \frac{1}{sechu} = coshu$$

Now lay off OL = ON = coshu. Also erect IK perpendicular to OO¹ and equal to OA. Then let OK meet the perpendicular to OO¹ at L in the point P. Hence

$$\frac{LP}{OL} = \frac{LP}{coshu} = \frac{IK}{OA} = \frac{tanhu}{1},$$

giving LP = sinhu. Hence the point P (coshu, sinhu) lies on the rectangular hyperbola x² - y² = 1, since cos²hu - sin²hu = 1. Now lay off OT = LP = sinhu. Then, taking V, the inverse of T with respect to the unit circle, we have

$$OV = \frac{1}{OT} = \frac{1}{sinhu} = cschu$$

It follows immediately that the line VP, coshu · x - sinhu · y = 1, is the tangent to the rectangular hyperbola at the point P. Here u represents double the sector AIP.

Now O¹Y = OZ - OW. Also

$$\sec \phi = \csc \theta = \frac{OE}{AE} = \frac{1}{sechu} - \frac{1}{\sqrt{1 - \tan^2 hu}} \quad 1$$

Then $O^1X \cdot \sec \phi = OX \cdot \sec \phi - OO^1 \cdot \sec \phi$, which may be written $O^1Y = OX \cdot \cosh u - OO^1 \cdot \cosh u$. But

$$\frac{\sinh u}{\cosh u} = \tanh u = \frac{v}{c} \therefore v \cdot \cosh u = c \cdot \sinh u$$

Hence

$$x^1 = \cosh u \cdot x - \sinh u \cdot ct \dots \dots \dots (1)$$

Furthermore, $O^1F = OF - OO^1 = OC + CF - (OM + MO^1) = OC - OM$. Then $O^1F \cdot \sec \phi = OC \cdot \sec \phi - OM \cdot \sec \phi$, which may be written

$$O^1G = ct \cdot \cosh u - OR \cdot \sin \phi \sec \phi = ct \cdot \cosh u - OR \sinh u$$

Hence

$$ct^1 = \cosh u \cdot ct - \sinh u \cdot x \dots \dots \dots (2)$$

To find the relative invariant, we have $O^1G - O^1Y = YG = XC_1 + C_1F_1 = XC \sec \phi + CF \sec \phi = XC \sec \phi + MO^1 \sec \phi = XC \cdot \sec \phi + RK_1 \sec \phi = XC \cdot \sec \phi + RQ \cdot \sin \phi \sec \phi = XC \cdot \sec \phi + XC \cdot \tan \phi = XC \left(\frac{1 + \sin \phi}{\cos \phi} \right)$. Hence

$$ct^1 - x^1 = \frac{\alpha}{c} (c + v) \cdot (ct - x) \dots \dots \dots (3)$$

$$[\alpha = (1 - v^2/c^2)^{-\frac{1}{2}}]$$

Similarly $O^1G + O^1Y = O^1G + GD_1 = O^1E_1 - D_1E_1 = OG_1 - OW - OB = OB_1 \sec \phi - (OW + XZ) = OB_1 \sec \phi - (CJ + OX \cdot \tan \phi) = OB_1 \sec \phi - (OC \cdot \tan \phi + CB_1 \cdot \tan \phi) = OB_1 \sec \phi - OB^1 \cdot \tan \phi = OB_1 \left(\frac{1 + \sin \phi}{\cos \phi} \right)$. Hence

$$ct^1 + x^1 = \frac{\alpha}{c} (c - v) \cdot (ct + x) \dots \dots \dots (4)$$

From (3) and (4), we obtain the relative invariant

$$\frac{ct^1 - x^1}{ct^1 + x^1} = \left(\frac{c + v}{c - v} \right) \left(\frac{ct - x}{ct + x} \right) \dots \dots \dots (5)$$

To find the absolute invariant, we have $O^1G = MD = BJ$ (parallels included between parallels). Also $O^1Y = WZ$. Now $\overline{BJ}^2 - \overline{WZ}^2 = (OJ - OB)^2 - (OZ - OW)^2 = (\overline{OJ}^2 - \overline{OB}^2) - (\overline{OZ}^2 - \overline{OW}^2) = 2(OJ \cdot OB - OZ \cdot OW)$. Also

$$\frac{OZ}{OJ} = \frac{XZ}{CJ} = \frac{OB}{OW}$$

$$\therefore OJ \cdot OB - OZ \cdot OW = 0$$

Hence

$$\overline{O^1G}^2 - \overline{O^1Y}^2 = (\overline{OJ}^2 - \overline{CJ}^2) - (\overline{OZ}^2 - \overline{XZ}^2) = \overline{OC}^2 - \overline{OX}^2$$

Thus we have, showing the absolute invariance of the interval

$$\overline{ds}^2 \equiv (ct^1)^2 - (x^1)^2 = (ct)^2 - (x)^2 = \overline{ds}^2 \dots \dots \dots (6)$$

This is otherwise algebraically shown by equations (1) and (2), since $(ct^1)^2 - (x^1)^2 = (\cosh u \cdot ct - \sinh u \cdot x)^2 - (\cosh u \cdot x - \sinh u \cdot ct)^2 = (ct)^2 (\cos^2 hu - \sin^2 hu) - x^2 (\cos^2 hu - \sin^2 hu) = (ct)^2 - (x)^2$.

It is easy to see that

$$OM = \frac{v}{c} x = \tanh u \cdot x,$$

$$SZ = \frac{c}{\sqrt{1 - v^2/c^2}} \left(t - \frac{vx}{c^2} \right) = \cosh u \cdot ct - \cosh u \cdot \tanh u \cdot x$$

Since the equations are linear in the variables, we have the absolute invariant for an infinitesimal region

$$\overline{ds'}^2 \equiv (cdt^1)^2 - (dx^1)^2 = (cdt)^2 - (dx)^2 \equiv \overline{ds}^2 \dots \dots \dots (7)$$

(IV)

In the opinion of some scientists, the theory of relativity cannot as yet be said to rest upon entirely stable foundations. This, too, in spite of Einstein's recent assertion that no one doubts the justification in principle of the general theory of relativity. In regard to the famous Michelson-Morley experiment, which constitutes one of the foundation stones of relativity, Dr. W. B. Cartmel of the University of Montreal has recently devised a formula which completely accounts for the results obtained by all those who have tried this experiment during the last half-century. In particular, it accounts strikingly for the conclusions of the elaborate investigations conducted by Dr. Dayton C. Miller, of

the Case School of Applied Science.¹⁰ A determinative coefficient in Cartmel's formula for the fringe shift turns out to be equal to $1/400$ in Miller's case, which accounts for the latter's finding $1/400$ of the expected fringe shift and $1/20$ of the expected velocity. In the admirable paper, summing up the results of his prolonged experiments, Miller concludes that there is an absolute motion of the solar system as a whole; and that this cosmic motion of the earth has a velocity of 208 kilometers per second, directed toward the "southern apex" in the constellation Dorado, the Sword Fish, about 20° south of the star Canopus, the second brightest star in the heavens, located in the midst of the Great Magellanic Cloud.¹¹ The combination of the orbital motion of the earth at the rate of 30 kilometers per second, with the motion of 208 kilometers per second at right angles to its orbit, gives the earth, according to Miller and Cartmel, an absolute motion through space in a spiral path. These experiments and conclusions, if eventually confirmed, will give a serious blow to the theory of relativity which postulates that no purely terrestrial experiment can be devised to detect the motion of the earth in space.

Even the constancy of light-velocity in empty space, another basic postulate of relativity, is not as yet established in the definitive sense. The last experiment to determine the light-velocity, initiated by the late Professor A. A. Michelson and completed after his death by Pease and Pearson, showed wide fluctuations of two kinds, with an uncertainty of from 3,280 to 6,560 feet. The figure for light-velocity given by this latest experiment is 186,270.75 miles or roughly 299,774 kilometers per second. Scientists are still far from realizing Michelson's dream of eliminating the uncertainty down to the last mile.

(V)

Professor Leigh Page of Yale University has recently advanced a new conception of relativity, designed not to controvert Einstein's general principle of relativity, but to broaden the bases of the restricted theory.¹²

¹⁰ I am indebted to Dr. Cartmel for abstracts of his two papers delivered at a joint meeting of the American Physical Society and the Optical Society of America, February 21-22, 1936.

¹¹ Dayton C. Miller: "The ether-drift experiment and the determination of the absolute motion of the earth." *Reviews of Modern Physics*, vol. 5, no. 3, July, 1933. I am indebted to Dr. Miller for copies of this and a number of other papers bearing on this subject.

¹² L. Page: "A new relativity." Paper I. "Fundamental principles and transformations between accelerated systems." *Physical Review* 49, pp. 254-268. (February 1, 1936). Paper II. "Transformation of the electromagnetic field between accelerated systems and the force equation, *ibid.* 49, pp. 466-469. (March 15, 1936).

The restricted theory postulates the constancy of light-velocity in Euclidean empty-space; and deals with reference frames which have constant relative velocities in a straight line. Following the lead of Professor Milne of Oxford University, who in his new work¹³ dispenses with the undefinable concepts of rigid measuring rods and periodic clocks, Page bases his "New Relativity" upon the constancy of light-velocity in Euclidean empty space, together with the postulated conception of a particle-observer with the capacity for deciding absolutely the time of events occurring at himself. The intrinsic conception of correlativity, expounded by Milne, is an elucidation of Einstein's laconic but somewhat vague statement: "Alle Stellen des Universums sind gleichwertig."

Einstein's fundamental postulate is that the "physical interval" between two near-by events is an absolute invariant for all reference systems. Page's theory is revolutionary, in that he has discovered, in an effectively empty world, a new category of reference systems with Euclidean geometries and constant light-velocity, which have constant relative accelerations (in the relativistic sense); but for which the physical interval, contrary to Einstein's fundamental assumption, is not an absolute invariant. This new theory, applicable to microscopic rather than to macroscopic phenomena, offers the possible prospect of acquiring a better understanding of the motions occurring in the atom.

For these systems with constant relative accelerations (in the relativistic sense), the writer suggests the name "activist" systems as contrasted with the "inertial" systems, having constant relative velocities in a straight line, of Einstein's restricted relativity. For these "activist" systems, Page has developed formulas which are the analog of the Voigt transformation of restricted relativity. For these formulas the writer ventures to suggest the name, the "Page transformation," in honor of the discoverer.

In the remainder of this paper is given, for the first time, it is presumed, a geometrical interpretation of the Page transformation and the associated invariants. The idea back of this treatment is identical with that of the first part of this paper, namely an exhibition, in geometrical terms, of the fundamental properties and relationships of the "Page transformation." As might be expected, however, the geometrical properties in the two cases are entirely different. In the former, the

¹³ E. A. Milne: *Relativity, gravitation, and world-structure*. (Oxford, Clarendon Press, 1935).

relative invariant belongs to the finite region of the plane; whereas the "physical interval," which is an absolute invariant, holds for the infinitesimal as well as for the finite domain. In the latter, the relative invariant holds only for the infinitesimal region of the plane, whereas the absolute invariant holds only for the finite region.

(VI)

A problem which Page studies in his first paper concerns the properties of the linear reference systems adjoined to two synchronous particle-

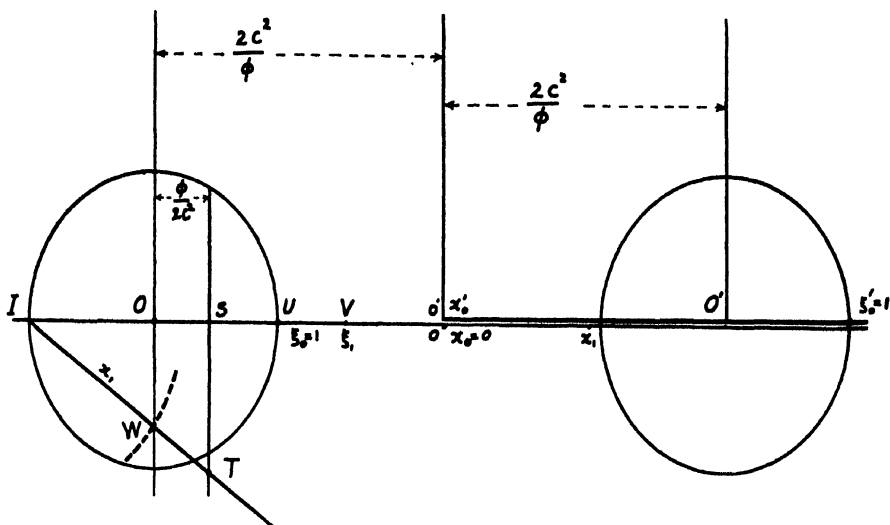


FIG. 2

observers P and Pⁱ which have a constant relative acceleration ϕ (in the relativistic sense). The differential equation of motion of Pⁱ relative to P is

$$\frac{d^2r}{dt^2} = (1 - v^2/c^2)^{1/2} \phi \dots \dots \dots (1)^{14}$$

The integral of this is readily seen to be

$$1 + \phi r/c^2 = (1 + \phi^2 t^2/c^2)^{1/2} \dots \dots \dots (2)$$

¹⁴ See, for example, J. Rice: Relativity. (Longmans, Green & Co., London and New York, 1923), equation (10), p. 60.

By making use of the basic equations of the Page theory, it is not difficult to arrive at the following transformation

$$x^1 = \frac{x \left(1 + \frac{\phi x}{2c^2} \right) - \frac{\phi t^2}{2}}{\left(1 + \frac{\phi x}{2c^2} \right)^2 - \frac{\phi^2 t^2}{4c^2}} \dots \dots \dots (3)$$

$$t^1 = \frac{t}{\left(1 + \frac{\phi x}{2c^2} \right)^2 - \frac{\phi^2 t^2}{4c^2}} \dots \dots \dots (4)$$

where (3) represents P¹'s estimation of the distance of an event Q, and (4) represents P¹'s "extended" time of the event Q. These are the formulas which will be referred to hereafter as the Page transformation by analogy with the Voigt transformation.¹⁵

It is now proposed to give a complete geometric representation and elucidation of this transformation, which is not attempted in Page's original paper. It is obvious that the origins of the two systems are coincident at the moment from which times in each are counted. The following substitutions are made for the sake of simplification:

$$\left. \begin{aligned} \xi &= 1 + \frac{\phi x}{2c^2}, & T &= \frac{\phi t}{2c} \\ \xi^1 &= 1 - \frac{\phi x^1}{2c^2}, & T^1 &= \frac{\phi t^1}{2c} \end{aligned} \right\} \quad (5)$$

In the two systems, S, S¹, this is equivalent to taking new origins at the points $-\frac{2c^2}{\phi}$ and $\frac{2c^2}{\phi}$ respectively, and combining with the latter a change in the sense of the axis. It is to be noted that when $x = 0$, $x^1 = 0$, $t = 0$, $t^1 = 0$, we have $\xi = 1$, $\xi^1 = 1$, $T = 0$, $T^1 = 0$.

Corresponding to a given point (event), say Q, represented in Fig. 2 with the abscissa x , and carrying the time co-ordinate t , for the S system, are the co-ordinates ξ , and T , in the transformed system. First let us carry out the transformations of S and S¹ to systems with new origins. Clearly we have directly.

¹⁵ L. Page: I, *ibid.*, pp. 260 f.

$$\frac{2c^2}{\phi} (-\xi^1 + 1) = \frac{x\xi - ct \cdot T}{\xi^2 - T^2}$$

$$\therefore \xi^1 = \frac{\xi^2 - T^2 - \frac{\phi}{2c^2} x \cdot \xi + \frac{\phi}{2c} t \cdot T}{\xi^2 - T^2} = \frac{\xi^2 - T^2 - (\xi - 1) \xi + T^2}{\xi^2 - T^2}$$

or

$$\xi^1 = \frac{\xi}{\xi^2 - T^2} \dots \dots \dots (6)$$

and the inverse

$$\xi = \frac{\xi^1}{\xi^{12} - T^{12}} \dots \dots \dots (7)$$

Similarly,

$$T^1 = \frac{T}{\xi^2 - T^2} \dots \dots \dots (8)$$

and

$$T = \frac{T^1}{\xi^{12} - T^{12}} \dots \dots \dots (9)$$

This transformation, either from (6) and (7) or from (8) and (9), gives

$$(\xi^2 - T^2) \cdot (\xi^{12} - T^{12}) = 1 \dots \dots \dots (10)$$

It yields, from (6) and (8) or from (7) and (9), the absolute invariant

$$\frac{\xi^1}{T^1} = \frac{\xi}{T} \dots \dots \dots (11)$$

Furthermore, it yields a relative invariant, which may readily be found. Thus from equations (5) we have

$$dx^1 = -\frac{2c^2}{\phi} d\xi^1, \quad dt^1 = \frac{2c}{\phi} dT^1$$

$$dx = \frac{2c^2}{\phi} d\xi, \quad dt = \frac{2c}{\phi} dT$$

Hence

$$c^2 dt^1 - dx^1 = \frac{4c^4}{\phi^2} (dT^1 - d\xi^1)$$

and

$$\begin{aligned} c^2 dt^2 - dx^2 &= \frac{4c^4}{\phi^2} (dT^2 - d\xi^2) \\ \therefore \frac{c^2 dt^1 - dx^1}{c^2 dt^2 - dx^2} &= \frac{dT^1 - d\xi^1}{dT^2 - d\xi^2} \dots\dots\dots (12) \end{aligned}$$

Now from (6) and (8) we have

$$\begin{aligned} dT^1 &= \frac{(\xi^2 + T^2) \cdot dT - 2\xi T d\xi}{(\xi^2 - T^2)^2} \\ d\xi^1 &= \frac{-(\xi^2 + T^2) d\xi + 2\xi T dT}{(\xi^2 - T^2)^2} \end{aligned}$$

Hence

$$dT^1 - d\xi^1 = \frac{dT^2 - d\xi^2}{(\xi^2 - T^2)^2} \dots\dots\dots (13)$$

From (10) and (13) we have the relative invariant

$$\frac{dT^1 - d\xi^1}{T^1 - \xi^1} = \frac{dT^2 - d\xi^2}{T^2 - \xi^2} \dots\dots\dots (14)$$

which, by means of (3), (4), and (12) may be written

$$\frac{c^2 dt^1 - x^1}{\left(\frac{\phi}{2c} t^1\right)^2 - \left(1 - \frac{\phi x^1}{2c^2}\right)^2} = \frac{c^2 dt^2 - dx^2}{\left(\frac{\phi}{2c} t\right)^2 - \left(1 + \frac{\phi x}{2c^2}\right)^2} \dots\dots\dots (15)$$

Since the physical interval $c^2 dt^{12} - dx^{12}$ is not an absolute invariant, Page's theory is in disagreement with the fundamental postulate on which Einstein's theory of relativity is based.

Now given x_1 and its associated time t_1 , the first problem is to find their correspondents ξ_1 and T_1 in the system transformed by the two equations (5), top line. Laying off the distance $\frac{2c^2}{\phi}$ to right and left of the common origin $O(O^1)$, we find the inverse S of this point $O(O^1)$ with reference to the unit circle with center O and radius OU . Describe

an arc, having $IW = x_1$ as radius and center I, and cutting vertical through S at the point T. Then

$$WT:IW::OS:OI$$

or

$$WT:x_1::\frac{\phi}{2c^2}:1$$

and therefore

$$WT = \frac{\phi}{2c^2} x_1 \dots \dots \dots (16)$$

Laying off $UV = WT$, we have $OV = OU + UV$, which gives, by (3),

$$OV = \xi_1 = 1 + \frac{\phi x_1}{c^2} \dots \dots \dots (17)$$

Constructing $\frac{\phi}{2c}$, knowing the value of c , we can in similar fashion find

$$T_1 = \frac{\phi}{2c} \cdot t_1, \text{ from the given value of } t_1.$$

Let us now transfer our attention to Fig. 3. We wish to give a complete geometrical interpretation of the Page transformation, given the co-ordinates of a point (ξ, T) . Given two lines OU, OP^1 at right angles to each other. Then lay off, on OP^1 , $OS = T$; and from S describe an arc with radius $= \xi$, cutting OU at P, giving $SP = \xi$. Draw the unit circle with center at O and cutting the horizontal and vertical lines through O in W and I. Construct the inverse, P_1 , of P with respect to the unit circle. Join I to P and on the vertical through O lay off $OP^1 = OP$. Through P^1 draw a line parallel to IP cutting horizontal through O in the point U. Construct the inverse U_1 of U with respect to the unit circle.

Now

$$OP = \sqrt{\overline{SP^2} - \overline{OS^2}} = \sqrt{\xi^2 - T^2}.$$

Also

$$OI:OP^1::OP:OU$$

$$\therefore OU = \overline{OP^2} = \xi^2 - T^2$$

Now join S to W, and through U_1 draw a line parallel to WS, cutting OP^1 at V. Draw straight lines VP_1 , VW, and SU. Then $VP_1 = \xi^1$ and $OV = T^1$.

The proof follows below. Since U_1V is parallel to WS,

$$\frac{OS}{OV} = \frac{OW}{OU_1} = \frac{1}{\overline{OP}_1^2} = \overline{OP}^2$$

$$\therefore \frac{OS}{OP} = OV \cdot OP = \frac{OV}{OP_1}.$$

Hence VP_1 is parallel to SP . Also

$$OV \cdot OW = OS \cdot OU_1 = OS \cdot \overline{OP}_1^2 = \frac{OS}{\overline{OP}^2} = \frac{OS}{OU}$$

or

$$OV:1 = OS:OU, \quad \text{since} \quad OW = 1.$$

Therefore $OV:OW = OS:OU$, showing that VW is parallel to SU .

Now

$$\frac{OS}{\overline{OV}} = \frac{SP}{\overline{VP}_1} \quad \text{or} \quad \frac{T}{\overline{OV}} = \frac{\xi}{\overline{VP}_1} \dots \dots \dots (18)$$

Also

$$\frac{OS}{\overline{OV}} = \frac{OU}{\overline{OW}} = \frac{\overline{OP}^2}{1},$$

and hence

$$\frac{T}{\overline{OV}} = \xi^2 - T^2 \dots \dots \dots (19)$$

We have

$$\overline{OP}^2 \cdot \overline{OP}_1^2 = 1$$

and hence

$$OU \cdot OU_1 = 1,$$

giving

$$(\xi^2 - T^2)(\overline{VP}_1^2 - \overline{OV}^2) = 1 \dots \dots \dots (20)$$

Also

$$\frac{OV}{OS} = \frac{OW}{OU} = \frac{OU_1}{1} = \overline{OP}_1^2 = \overline{VP}_1^2 - \overline{OV}^2$$

$$\therefore \frac{OV}{T} = \overline{VP}_1^2 - \overline{OV}^2 \dots \dots \dots (21)$$

Setting $VP_1 = \xi^1$, $OV = T^1$, we have from (18)

$$\frac{T}{T^1} = \frac{\xi}{\xi^1} \dots \dots \dots (11)$$

From (19) we have

$$\frac{T}{T^1} = \xi^2 - T^2 \dots \dots \dots (8)$$

From (20), we have

$$(\xi^2 - T^2)(\xi^1 - T^1) = 1 \dots \dots \dots (10)$$

and from (21) we have

$$\frac{T^1}{T} = \xi^1 - T^1 \dots \dots \dots (9)$$

Hence from equations (8), (10), and (11) we have

$$\xi^1 = \frac{\xi}{\xi^2 - T^2} \dots \dots \dots (6)$$

and

$$\xi = \frac{\xi^1}{\xi^1 - T^1} \dots \dots \dots (7)$$

Now from the method of construction we can easily see that if we give increments to ξ and T so that the line representing $\xi + d\xi$ is parallel to SP , we shall find the line representing $\xi^1 + d\xi^1$ to be parallel to VP_1 and consequently parallel to SP . From the resulting similar triangles, six in all, we have

$$\frac{T^1 + dT^1}{T^1} = \frac{T + dT}{T} \quad \text{or} \quad \frac{dT^1}{T^1} = \frac{dT}{T}$$

Also

$$\frac{dT^1}{d\xi^1} = \frac{dT}{d\xi} = \frac{T^1}{\xi^1} = \frac{T}{\xi}.$$

$$\therefore \frac{\overline{dT^1} - d\xi^1}{d\xi^1} = \frac{\overline{dT^2} - d\xi^2}{d\xi^2}$$

Hence

$$\frac{\overline{dT^1} - d\xi^1}{\overline{dT^2} - d\xi^2} = \frac{d\xi^1}{d\xi^2} = \frac{dT^1}{dT^2} = \frac{T^1}{T^2} = \frac{\xi^1}{\xi^2} = \frac{T^1 - \xi^1}{T^2 - \xi^2}$$

and

$$\frac{\overline{dT^1} - d\xi^1}{T^1 - \xi^1} = \frac{\overline{dT^2} - d\xi^2}{T^2 - \xi^2} \dots\dots\dots (14)$$

Thus, starting with x_1 , t_1 we can find ξ_1 , T_1 from Fig. 2; and from ξ_1 , T_1 we can find ξ_1^1 , T_1^1 from Fig. 3. Then ξ_1^1 can be laid off from O^1 , center of the unit circle at the right of Fig. 2; and with it is associated the time T_1^1 .

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SOME PRIMITIVE MOSS-MITES OF NORTH CAROLINA

By ARTHUR PAUL JACOT

PLATE 1

The moss-mites are regarded by the Germans as a supercohors of the suborder Sarcoptiformes (10, p. 95). The supercohors Oribatei is divided by them into twenty families. In the last edition of Pratt (8, p. 516) the moss-mites are presented by Ewing as the Galumnidae (casting out the term Oribata). I have found a middle course more convenient, placing the entire group, as Banks did, in the superfamily Oribatoidea.

In 1932 Trägårdh (9) described a new species of primitive mite which he secluded in a new suborder. A few months later Grandjean (2) described two related species, and pointed out that Berlese had already described three others, and that these mites were all characteristically moss-mites though presenting certain primitive characters.

I now present my more simple classification, applicable to the genera of the eastern United States and Europe. It will be noted that some of the groups I formerly regarded as families are now reduced to subfamilies, while subfamilies are reduced to tribes. The tendency in Europe has been to make up accessory groups above family rank introducing such terms as subphalanx, phalanx, superphalanx, subcohors, cohors, and supercohors. My course is to abolish these groups foreign to systematic zoölogy and use the group name tribe, so common and satisfactory in entomology. Moreover some of these groups are highly artificial. For instance the family Nanhermanniidae is based on the fusion of the adanal covers with the notogaster (3, p. 20). The genus *Trhypochthoniella* bridges the *Trhypochthoniinae* and the *Malaconothridae*. I see no reason for placing *Gustavia* and *Zetorchestes* in special families any more than *Suctobelba*, *Pelops* and others. Other genera have since been found which bridge many of the gaps which formerly were thought to demark the larger groups. All group names are formed from the oldest valid genus within that group, and it stands for the type of the group.

Grandjean is now working on the phylogeny of the Oribatoidea.

Although his results are of considerable interest they do not at present promise to be practical. The present arrangement claims to be simple and easily handled, without regard to evolutionary relations.

Superfamily ORIBATOIDEA

Tyroglyphina (4, p. 208) with a pair of specialized bristles (pseudo-stigmatic organs) on posterior part of cephaloprothorax each springing from cuplike depression (pseudostigmata), or with large anal and genital apertures each of which is closed by at least one pair of sclerotized plates, or with both

Type: *Oribata* (6, p. 65)

Key to Families (Adults)

- 1 Legs capable of being drawn into anterior part of notogaster, and covered by the cephalon (aspis) *Phthiracaridae*
1. Legs incapable of such retraction .. 2
- 2 Form elongate; parasterna IV nearly as long as broad; genital aperture so posterior in position (by the great expansion of parasterna IV) that ovipositor is directed posteriad within the abdomen, near the anal aperture *Eulohmannidae*
2. Parasterna IV slender; genital aperture near parasterna IV; ovipositor directed anteriad within the body . *Oribatidae*

I had formerly recognized the *Epilohmannidae* but find that *Eulohmannia* belongs in this family and has precedence, thus necessitating the change in family name

Key to Subfamilies and Tribes of *Oribatidae* (Adults)

- 1 Mandibles projecting conspicuously beyond rostrum; without distinctly differentiated notogaster *Parhypochthoninae*
1. Mandibles covered by rostrum, or, abdomen with a distinct notogaster 2
2. Anal and genital apertures occupying most of the abdomen behind parasterna IV, broadly contiguous, the anal aperture usually narrowing posteriad through the progressive narrowing of the V-shaped ventral plate 3
2. Anal and genital apertures smaller, more or less quadrate, usually widely separated, sometimes touching, if apertures are broadly contiguous then legs are long and very slender 5
3. Dorsum of abdomen with one or more transverse divisions *Hypochthoninae*
3. Dorsum of abdomen without transverse divisions *Camisiinae* 4
4. Integument pale *Lohmannium*
4. Integument brown to black *Camisiini*
5. Notogaster without lateral expansions, anterolateral corners sometimes produced as an outjutting boss, ridge or blade * 6
5. Notogaster with thin, lateral expansions which are usually bent ventrad to cover at least the leg insertions *Galumninae* 8

6. Notogaster smooth, if granular then lamellae are absent to poorly developed
Oribatinae 7
6. Notogaster variously sculptured, if granular then lamellae are well developed
*Cepheinae**
7. Lamellae absent or developed as low ridges or nobs..... Oribatini
7. Lamellae developed as blades, free along lateral edge..... Gustaviini
8. Mandibles styliform or chelae minute; notogaster roughed by accumulation
of a coating of extraneous matter or otherwise..... Pelopini
8. Mandibles broad throughout, chelae massive; notogaster smooth..... 9
9. Lamellae attached to cephaloprothorax only at their base and posteromesal
edges, and developed as broad blades with median edge free or more or less
fused, covering most of the cephaloprothorax..... Achipteriini†
9. Lamellae not so highly developed..... 10
10. Pteromorphae produced far anterior of abdomen even to ends of lamellae, or
joined over the cephaloprothorax to form a broad bridge..... 11
10. Pteromorphae not extending far anterior of abdomen..... Ceratozetini
11. Pteromorphae joined to each other along anterior edge of abdomen, forming
more or less of a bridge over the cephaloprothorax..... Oripodini
11. Pteromorphae very large, extending downward to cover part of ventral plate
and forward to cover part of cephaloprothorax; legs received in cupboards
in sides of abdomen and entirely enclosed by the pteromorphae; lamellae
small to absent..... Galumnini

* *Cepheus* has page precedence over *Carabodes*.

† *Achipteria* has precedence over *Oribatella*.

The following are new to science. The types will be deposited at the National Museum.

Genus *GEHYPOCHTHONIUS* gen. nov.

Parhypochthoniinae without apophyses on sides of abdomen, with one transverse suture across dorsum of abdomen, bihamate ungues, anal covers with two bristles each, paranal covers with three bristles each, genital covers with six median and three lateral bristles.

Type: *Gehypochthonius rhadamanthus* sp. nov.

Gehypochthonius rhadamanthus sp. nov.

Figures 1 and 2

Animal entirely white or glassy (resembling an immature); size: total length (including mandibles) 0.3 mm., greatest width 0.09 mm., distance between pseudostigmata 0.04 mm.; dorsal bristles rather short (shorter than tarsus I), straight, of equal caliber throughout; rostral bristles borne on a small, triangular projection (the rostrum) which

sets at a lower level than anterior edge of cephaloprothorax; major exopseudostigmatic bristles as long as rostral, inserted fairly close to pseudostigmata; minor exopseudostigmatic bristles very short, inserted below pseudostigmata; mandibles broadly exposed, with a depressed bristle over the chela and one farther back (figure 1); palps four segmented, not including base (figure 1), their tarsus with a curved spine on dorsal face; pseudostigmata with well formed rim; pseudostigmatic organ longer than longest bristle, head swollen, armed with four short, stout bristles, in each of four longitudinal rows, spaced almost their length from each other.

Notogaster divided by transverse fold; anterior section with twelve bristles arranged in two transverse rows, a3 slightly anterior to the others; posterior section with sixteen bristles on dorsal aspect, and two on ventral face, the dorsal bristles arranged in three transverse rows the ends of which curve anteriad. Thus the species is *meritrich* (totaling only thirty notogastral bristles) (3, p. 22).

Bristles of anogenital area arranged as in figure 2. Apodemata III and IV each with three bristles (figure 2); I am able to see but two bristles on apodemata II and three on apodemata I, in the none too satisfactory material before me. Legs I as in figure 1; tarsi I with a long, curved spine springing from near proximal end (as in so many soil Oribatidae). Tarsal hooks fairly large, subequal, with a minute point between their proximal ends.

Thus this species has the bristle arrangement and pseudostigmatic organs characteristic of the *Hypochthoniinae* but the uncovered mouth parts of the *Parhypochthoniinae*. I place it in the latter subfamily as a highly specialized species approaching the *Hypochthoniinae*. It is capable of considerable extension and telescopic retraction at the mid-thoracic region. The figure shows the animal very much retracted.

Material examined: Twenty-five specimens from soil, two to five inches deep, of *Andropogon* sod (upper inch removed), old-field abandoned twelve years, Bent Creek, Pisgah Forest, North Carolina; taken February 6th, 1935, slide 34F23.2-5 (*cotypes*). Five specimens from lower part of F-layer of isolated, pastured and trampled forty-year-old, short-leaf pine stand, two miles beyond Bent Creek on the Asheville-Brevard road; taken October 15th, 1934, slides 34F10.3P1, -P2, and -R2. One specimen from earth of close-cropped pasture sod near the preceding, slide 3453A. One specimen from soil of eighty-year-old pine-oak woodland, Bent Creek Experimental Forest; taken January 7th, 1935, slide 34F21-10. Six specimens from a root one inch in diam-

eter and six inches long, four inches below the surface, same spot as preceding, slide 34F21r2.

This species is thus distinctly a dweller in the soil and I take particular pleasure in naming it after Rhadamanthus, one of the judges of the underworld.

Genus *EOBRACHYCHTHONIUS* gen. nov.

Hypochthoniinae resembling *Brachychthonius* but with ventral plate divided by a transverse suture, and with four small plates along sides below the notogastral plates (figures 3 and 4).

Type: *Eobrachychthonius sexnotatus* sp. nov.

Eobrachychthonius sexnotatus, sp. nov.

Figures 3 and 4

Color rather bright yellow, rostrum colorless; size of a female: length 0.27 mm., breadth 0.197 mm.; bristles medium long, fine, tapering; major exopseudostigmatic bristles as long as lamellar, inserted on a sclerotized ring (figures 3 and 4) which projects well beyond surface of cephaloprothorax; minor exopseudostigmatic bristles indicated by an insertion; pseudostigmatic organ clavate, studded with short bristles (figure 3); center of vertex with six symmetrically spaced areoles; two or three others grouped about exopseudostigmatic bristle ring.

Notogaster nearly as broad as long, much broader than cephaloprothorax, anterior edge with a small projection; bristles a2, b2, and b3 inserted on periphery of plate below a sclerotized ridge; a3 inserted on anteriormost lateral plate; bristles c2, d2, and e2 also situated at edge of their plates below a sclerotized ridge; other bristles and areoles disposed as in figures 3 and 4. A bristle near posterior corner of posterior ventral plate. If the lateral plates and the two ventral are regarded as parts of a notogaster and of a ventral plate which have become fragmented, the species is more specialized than species of *Brachychthonius* because the fragmentation has gone further. If these additional plates are regarded as parts of a fragmented notogaster then the three plates of the dorsum are also parts of a fragmented notogaster and thus do not indicate a primitive segmented condition. It seems more reasonable, however, to adopt the view that all these plates are of primitive origin. Since dorsal plate I-II subtends two laterals, these two laterals seem to corroborate the view that dorsal plate I-II (the post-thoracic) is formed of two fused transverse plates. Moreover there is a single plate beneath

dorsal plate III and a small plate beneath dorsal plate IV. To be consistent, the two ventral plates must likewise be regarded as a primitive condition and consequently that the ventral plate of the higher oribatoids is made up of two plates and that the bristle thereon is a ventral plate bristle.

Bristles of ventral aspect as usual in this subfamily but anal covers with their two bristles inserted on anterior half; genital covers with the anterior bristle of lateral row inserted quite close to median row. Apodematal bristles as in figure 4.

Palps four segmented (in addition to basal segment).

The females seem to bear but one egg at a time.

Cotypes: Eleven specimens from *Andropogon* sod, from near top of Glen Bald, Bent Creek Experimental Forest; taken April 17th, 1935, slide 34F31-10.

Eobrachychthonius latus **comb nov.** (1, p. 220, pl. 19, fig. 38) from Lake City, Florida, differs by having four areoles on vertex and, if the figure is accurate, no other areoles, no rostral bristles (!), and notogastral bristles slightly differently distributed. As Berlese has omitted the bristles of lateral plate I and bristle c2 (of dorsal plate III), the absent rostrals may also be an oversight.

An only specimen from grass cover of a hill at Marshall, Illinois, taken January 2nd, 1933, by Frison and Mohr, slide 33IS1/2 # 19d, is identical except that it lacks the areoles.

***Hypochthonius rufulus carolinicus* subsp. nov.**

Differs from the species in that the pectinations of the pseudostigmatic organs number from fifteen to nineteen (even to twenty-three) while those of the species (of Europe) number from six to seven.

In 1934 (5, p. 260) I described a northern subspecies characterized by having but three to four pectinations. I also stated that insertions of bristles c1 were on the same transverse plane as c2 or more posterior. I now find that this character is not as constant or definite as might be desired. There is however, another character which is more obvious and seems to be quite constant, namely, the insertions of bristles b2 are about half way between b1 and b3 (though more posterior) while in the species the insertions are much nearer b3. This difference holds for both of the American subspecies, and for *Hypochthonius luteus* (7, p. 343).

The notogastral bristles of *H. r. carolinicus* are closely, more conspicuously, and more roughly barbed than in the species.

Material examined: Six specimens from litter of old-field woodland, Bent Creek Experimental Forest; taken September 20th, 1934, slides 34F4.3-5 (*cotypes*) and 34F4.1-19.

APPALACHIAN FOREST EXPERIMENT STATION,
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PLATE 1

Gehypochthonius rhadamanthus gen. et sp. nov.

Fig. 1. Lateral aspect, legs II to IV omitted; \times 466.

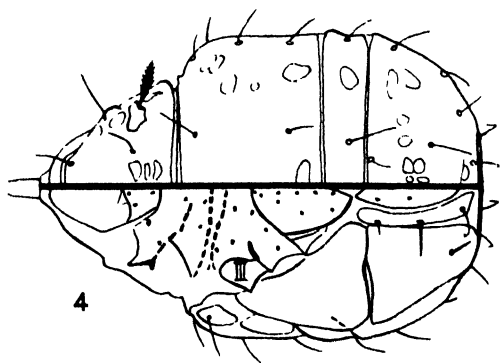
Fig. 2. Ventral face of abdomen; free hand.

Eobrachychthonius sexnotatus gen. et sp. nov.

Fig. 3. Lateral aspect, legs II to IV omitted; \times 376.

Fig. 4. Dorso/ventral aspects, legs and mouth parts omitted; \times 285.

PLATE 1



A NEW MULTIPLE CONSTANT TEMPERATURE APPARATUS FOR EXPERIMENTAL WORK IN BIOLOGY¹

By R. E. COKER, Professor of Zoology
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University of North Carolina

PLATES 2, 3, AND TWO TEXT FIGURES

The maintenance of precise constant temperatures at levels above room temperature presents relatively little difficulty, but when temperatures below or about the general level of room temperatures are required, the problem is much more complex. It is not readily solved by the use of "cold rooms," except as one may set heat-controlled baths within a cold room having a temperature below the minimum to be maintained in the several baths. There are several types of apparatus in which a ladder-like series of constant temperatures is maintained between fixed extremes, but in most of these the intermediate temperatures are not subject to strict individual control.² In connection with experimental work with Entomostraca it was desirable to have an apparatus to meet the following qualifications: (1) It should possess several similar chambers; (2) in each chamber the temperature should be maintainable at any desired level without regard to room temperature or to the temperatures in other chambers; (3) low or high temperatures within the range of metabolic activity of the animals should be available; (4) constancy for indefinite periods should be possible; and (5) precision to a fraction of a degree at least should be attainable. It was desirable also that the apparatus should be compact enough to be kept in a room of ordinary size, that it should be capable of being moved if necessary and that it should not be too expensive. A series of constant temperature rooms with several refrigerating units was beyond the

¹ The construction of the apparatus was made possible by a grant from the Rockefeller Fund for Research in Pure Science in the University of North Carolina. We are glad to acknowledge the courtesy of the Department of Chemistry of the University of North Carolina in permitting the building of the apparatus in its shop.

² See Coker, R. E., "Reactions of some freshwater copepods to high temperatures," in *Journal of the E. M. Sci. Soc.* 50: 143-159, 1934, and references therein cited.

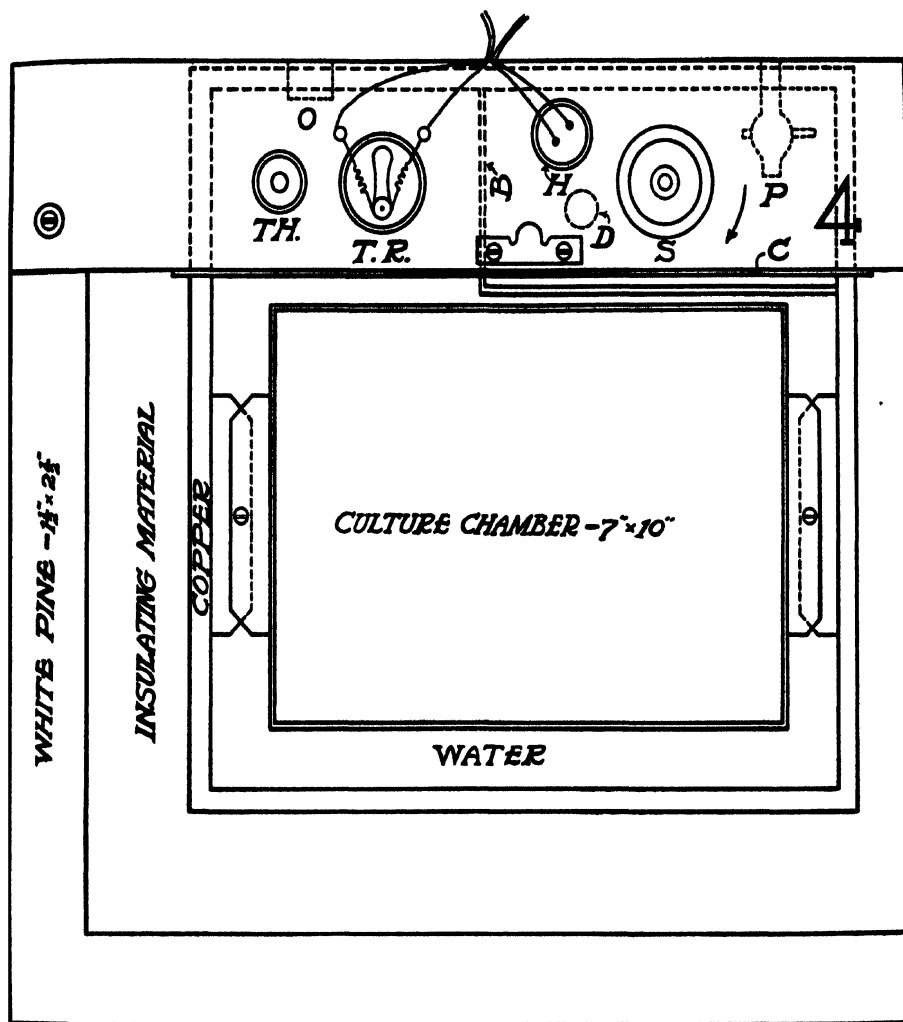
range of feasibility and a single low temperature room housing several heated baths was also impracticable.

The solution of the problem was sought in a multiple chamber apparatus, each chamber to be well insulated, supplied with water circulated from a single refrigeration chamber and equipped with its individual heating and control units. The apparatus designed and constructed is here illustrated and briefly described. The means available made it necessary to limit the number of operating chambers to six, but the design will permit a greater number if desired.

Our apparatus as a whole is 6' 4" long, 3' wide and 16½" high (or 24" to top of motor). To make the doors of the chambers convenient of access the apparatus is set on a skeleton stand 21" high, so that the top is about 38" above the floor. The size, exterior design and finish are such as to make a presentable piece of laboratory furniture (pl. 2). Actually we have the compressor of the refrigerating unit set on the floor nearby (out of view in pl. 2), but it could as well be placed on a base attached to the support and beneath the chambers, if this were desirable in the interest of compactness and appearance. To remove the apparatus from one room to another it would be necessary only to disconnect two electric lines, drain out the water into tubs, transfer the apparatus in two or three parts (three by the present plan of installation), refill from the tubs, and make new connections to electric outlets in the second room. In other words, the apparatus can be moved with little more difficulty than is involved in moving a large domestic electric refrigerator, although it occupies more floor space than the latter.

The photographic illustrations (pls. 2 and 3) and the plan (figs. 1 and 2) make an extended description unnecessary. In one end is a copper-lined refrigeration chamber, or reservoir 27" x 18" x 12", interior dimensions, containing about 20 gallons of water and glycerine mixture. The glycerine is added to prevent formation of ice on the refrigerating coils and to make possible a bottom temperature below 4°C., which is not obtainable without the use of an anti-freeze mixture. At present the mixture is about 11% glycerine. The temperature in the reservoir is thermostatically controlled, an automatic expansion valve governing the compressor. Accurate control of temperature in this chamber is unnecessary, but the bottom temperature here should be a little below the minimum desired in any operating chamber.

An electrically-driven centrifugal automobile pump circulates water from the bottom of the reservoir through a pipe running through a median conduit to the other end and having a lateral outlet to each operating chamber. On each of these outlets, and within the copper-



TOP VIEW (LID REMOVED)

TEXT FIG. 1. PLAN OF CHAMBER NO. 4, TOP VIEW WITH LID REMOVED: PETCOCK (P), DRAIN (D), OVERFLOW (O), AND WALL OF DRAFT TUBE (B) SHOWN IN PROJECTION

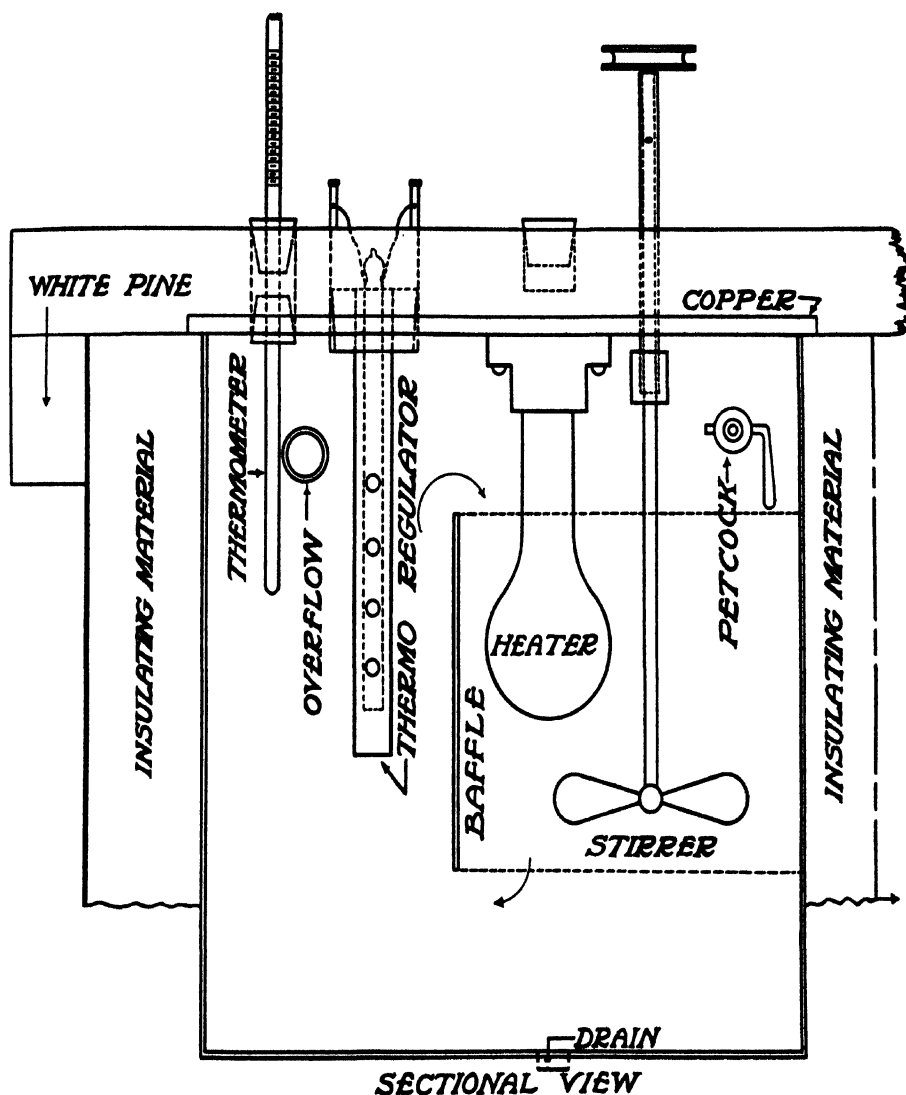
Th, socket for thermometer; *TR.*, thermoregulator; *S*, socket for stirrer shaft; *C*, copper lining reflected up; *H*, heater.

lined operating chamber (fig. 1 and pl. 3, below) supplied by it, there is an ordinary petcock (P)² which is regulated by hand. Strict calibration

² The letters in parentheses refer to corresponding letters in fig. 1.

of flow from the petcock is not requisite; it is only necessary that the flow should be at least adequate to compensate for the loss of heat by escape through the insulation of the chamber or otherwise, with a margin of safety. Actually the heat loss has been found to be much less than was expected, so that a drip, or at most a dribble, from the cock is generally all that is necessary. The proper discharge at the cock is readily determined by trial within a few minutes at the time the temperature in a given chamber is first fixed. Thereafter, the cock should require no attention. In practice we so adjust the flow that the heating unit, a submerged lampbulb, is not "off" for more than three to five minutes at a time. (It is "on" for 30-60 seconds at a time.) The excess water in the chamber discharges through a small overflow pipe (O) into a return main in the pipe conduit which leads back into the refrigerating reservoir at the end; thus, the same mixture is used over and over again, and whatever heat is acquired in the several chambers is removed in the reservoir. The heating unit in every operating chamber is a light bulb (fig. 2 and pl. 3, below) screwed into a socket (H) fixed in the ceiling of the chamber, the bulb itself being immersed in the bath liquid. The region around the bulb is fenced in by a copper partition (B) terminating, above, about $\frac{3}{4}$ inches below the surface of the liquid and, below, about 2 inches above the bottom of the chamber. Into this partially enclosed mixing space, or draft tube (see fig. 1 and pl. 3, below), falls also the drip of cold liquid from the petcock. Between the heater and the cold drip there is a propeller-type stirrer (S) of copper in continuous rotation at a speed of 600 r.p.m. Warm and cold water are thus promptly and thoroughly mixed and driven to the bottom of the draft tube to flow rapidly out in all directions over the bottom, up along the sides and back into the draft tube over the top of its wall. The several stirrers are driven by belts running from as many pulleys on a central shaft to pulleys at the top of each stirrer shaft (pl. 3, below). Both the main stirrer shaft and the pump in the refrigeration chamber are driven by individual belts from a common motor, a feature of safety, convenience and economy. We use a $\frac{1}{6}$ H.P. motor mounted on rubber to dampen vibration. Stoppage of electric current for any length of time would, of course, permit a slow fluctuation of temperature but this is a contingency to be faced with any electrically operated heating or cooling unit. It is significant that in this apparatus, interruption of current will cut off both heat and the circulation of the cooling medium. The insulation is then sufficient to maintain approximate constancy for a number of hours.

The temperature chamber proper, our culture chamber (fig. 1 and pl. 3, below, left) is a copper vessel, 10" x 7" x 9½", partially immersed



TEXT FIG. 2. SECTIONAL PLAN OF A SINGLE TEMPERATURE CHAMBER IN REAR PART

in the liquid of the operating chamber which is 12" x 12" x 12", and kept in place by a bolt on each side screwed through one copper flange attached to the outside of the wall of the temperature chamber near the

top and another attached to an inner wall of the operating chamber at the same level.

It is obvious now that the temperature of the air within each chamber is governed by that of the thoroughly mixed liquid surrounding it and that this in turn is controlled roughly by the inflow of cold mixture at the petcock and more precisely by the heating unit. The regulation of the operation of the heater is the primary consideration and will be explained in detail. First, however, it may be emphasized that the extensive use of copper in the lining of the chamber, in the wall of the draft tube, and in that of the temperature chamber proper, insures rapid conduction of heat to all parts, while the use of the electric lamp as a heating unit eliminates much of the lag that characterizes some heating coils.

The 110 volt heater is controlled by a relay which operates on a 6 volt, 6 milliampere circuit, this low current being necessary because of the delicacy of the mercury thermoregulator (TR), by which the relay in turn is controlled, immersed in the bath. The six relays, one for each chamber, are set up neatly in the control box (pl. 3, above), which for convenience is attached to the apparatus at the end opposite to the refrigeration reservoir (pl. 2). The low voltage current is derived from the regular lighting circuit through a small transformer, a rectifier and a condenser (reducing voltage and converting A.C. to D.C.). The plan of control is essentially as follows: When the capillary column of mercury in the thermoregulator of an operating chamber rises to the level of the electrodes, the low voltage circuit is closed, magnetizing the relay and opening the switch to break the 110 volt circuit and cut off the heater. When the capillary column of mercury falls the least bit, the low voltage circuit is broken, releasing the switch, and bringing the heater back into operation. The thermoregulators are readily adjustable to any desired temperatures.

The wiring plan is such that, save for the thermoregulators, all controls having to do with the heating units (the vital part of the mechanism as regards precision) are installed in the previously mentioned control box. This box 10" x 14" x 3" (pl. 2 and pl. 3, above) contains, therefore, a transformer, a rectifier, a condenser, 6 relays, and 6 small double-pole tumbler switches, each of the switches governing both a heating circuit (110 V) and a control circuit (6 V) for an individual chamber.

Precision in temperature control for any chamber is conditioned, first, by the efficiency of the thermoregulator, and those used are specified

by the manufacturers to have an accuracy within $0.1^{\circ}\text{C}.$; and, second, by the effectiveness of the mechanisms employed for the uniform distribution of temperature within the chamber. The second feature is the more difficult to measure, but it seems to be efficient within $0.1^{\circ}\text{C}.$

A contingency to be avoided is the burning out of a light bulb. We now employ bulbs with carbon filaments because of their greater heat generation (a quality associated with inefficiency in lighting). If the bulbs are changed once in two or three months there is little likelihood of failure of the heating unit.

Within ordinary limits the temperature of any one of the several chambers may be set at any level regardless of the temperature maintained in any of the other chambers and it may be changed at any time by adjustments that include merely a small alteration in the flow of water at the petcock and the resetting of the thermoregulator; such adjustments ordinarily require but a few minutes to effect. The temperatures we have needed to employ up to the present time have been between 9° and $30^{\circ}\text{C}.$ Merely for a trial, the senior author set one chamber at 51° and another immediately facing it at $-1.5^{\circ}\text{C}.$ There was no difficulty in maintaining constant temperatures of the water in the two compartments running simultaneously during a period of several days. In the low compartment ice formed on the floor of the culture chamber proper, but not in a beaker placed therein. We assume that with such extremes supplemental internal insulation would be required for precision of control, and presumably this could be arranged without special difficulty.

It is obvious that much depends upon many details of design and construction with reference to wiring, to the packing and lubrication of shafts passing through the top of the cabinet, to arrangements for packing the pump and preventing any leakage of oil from it into the reservoir, to the return of overflow from the several chambers, to insulation, fitting of covers, etc. To describe all of these details would extend the description to too great a length. For the apparently successful solution of the baffling problems of detail as well as for a large measure of the general design, credit should be given to the junior author.

We did not find on the market thermometers exactly adapted for use in the apparatus, but the Taylor Instrument Company has made for us some thermometers with a long stem and a scale reading in tenths of a degree from 0° to $40^{\circ}\text{C}.$, the lowest reading on the scale being about three inches above the upper limit of immersion. The space between

the upper limit of immersion and the bottom of the scale provides for passage through the insulation of the apparatus and the long stem makes the tenth degree scale marks sufficiently well spaced for precision in reading.

SUMMARY

There is described a multiple constant temperature apparatus in which the temperature of each chamber is quite independent of any other, in which temperatures below room temperature or above may be maintained with equal facility, in which precision to a fraction of a degree can be maintained for indefinite periods, and in which each chamber offers complete flexibility as to temperatures within a wide range.

PLATE 2

MULTIPLE CONSTANT TEMPERATURE APPARATUS

The compressor, on the floor beyond the apparatus, is not visible.

PLATE 3

- (Above) Interior of control box showing, *left*, rectifier, condenser, and transformer, and, *right center*, 6 relays and 6 trip-switches, with some of the connecting wires.
- (Below) Top view of portion of apparatus, with lids removed from chambers Nos. 4 and 5. The culture chamber has been removed from No. 5 to expose the draft tube enclosing, from right to left, heater, stirrer shaft, and petcock.

PLATE 2

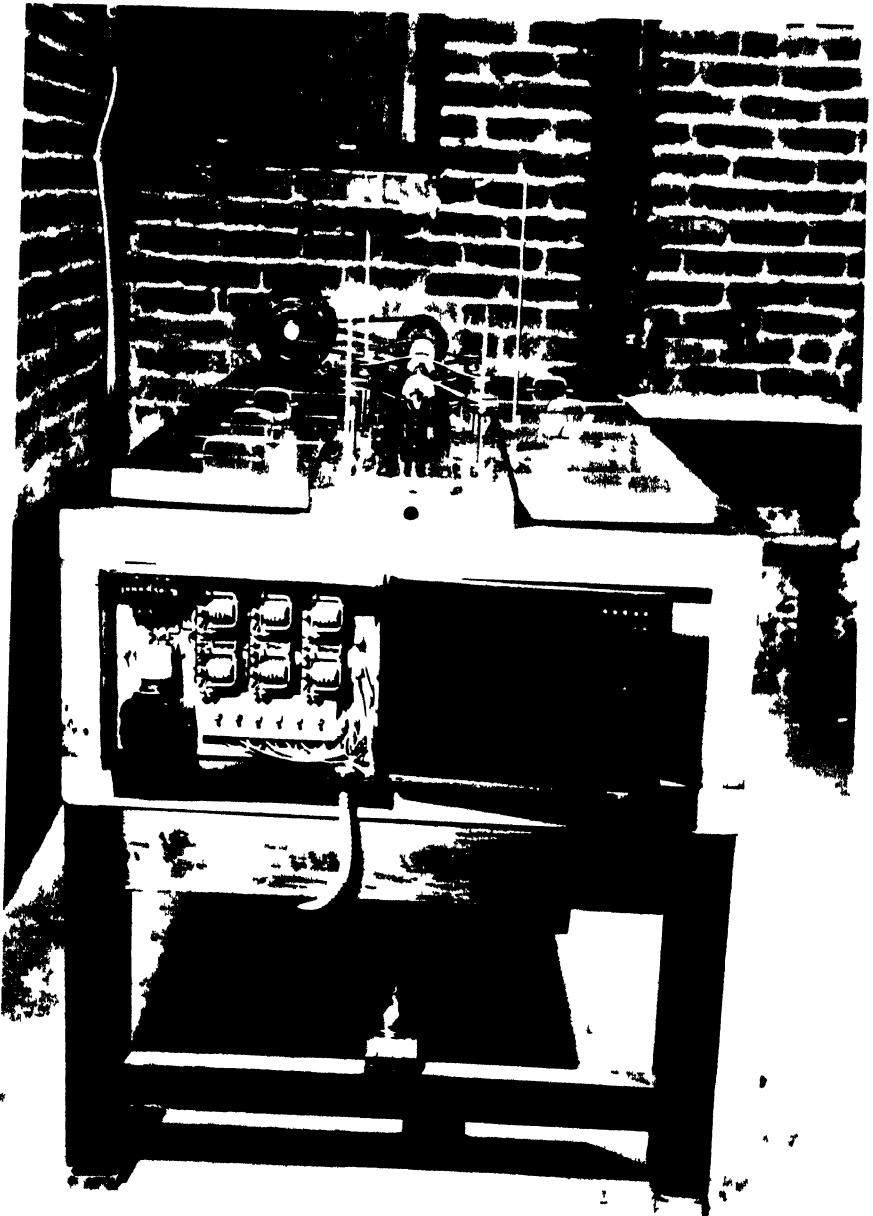
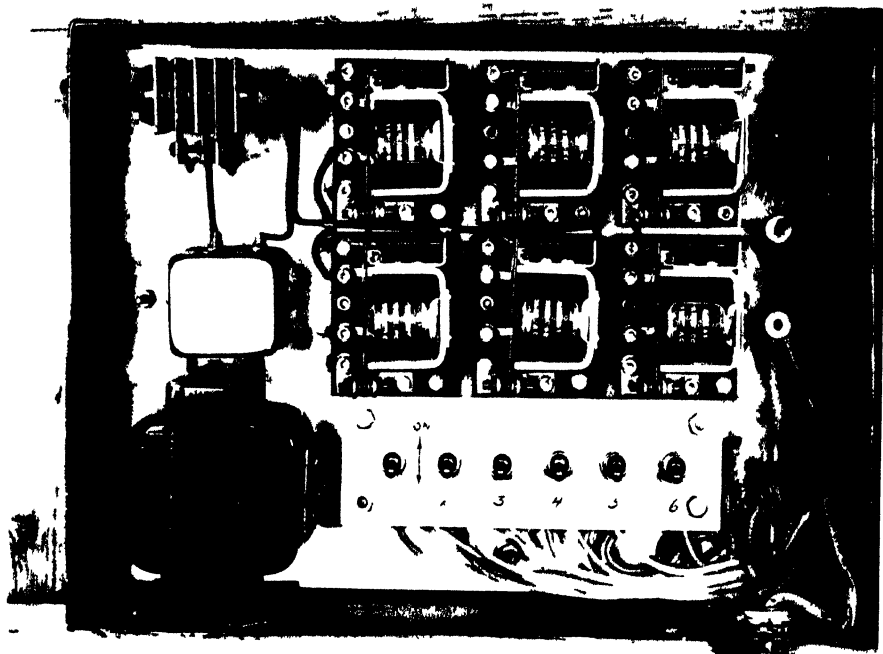


PLATE 3



RECENT FORAMINIFERA FROM NEAR BEAUFORT, NORTH CAROLINA

By WADE H. HADLEY, JR.

ONE TEXT FIGURE

The species of foraminifera listed below were obtained from shallow water dredgings and shore sands collected in the area of Beaufort, N. C., during the summer of 1933. The writer is indebted to the U. S. Bureau of Fisheries and to Dr. Herbert F. Prytherch, Director of the Bureau's Biological Station at Beaufort, N. C., for cooperation and the use of facilities which made these collections possible.

Stations from which material was collected for this study:

Sta. No. 1—Just west of sea buoy off Beaufort Inlet. Bottom sample taken in 40 feet of water.

Sta. No. 2—Directly off Atlantic Beach, one mile from shore. Bottom sample taken in 30 feet of water.

Sta. No. 3—Sand from Shackleford Beach, collected in the area between tides.

A species of *Bolivina* occurs in the dredgings from the Beaufort area which appears to be new and is here described as *Bolivina beaufortana* n. sp.

Species recorded from the above three stations

	Station		
	1	2	3
* <i>Quinqueloculina costata</i> d'Orbigny		R	
<i>Quinqueloculina</i> cf. <i>flexuosa</i> d'Orbigny	C	C	C
* <i>Quinqueloculina lamareckiana</i> d'Orbigny	C	C	
<i>Quinqueloculina poeyana</i> d'Orbigny	C	C	
* <i>Quinqueloculina</i> cf. <i>seminula</i> (Linnaeus)	C		C
* <i>Spiroloculina planulata</i> (Lamarek)		R	
<i>Guttulina spicaeformis</i> (Roemer) var. <i>australis</i> (d'Orbigny).	C	C	
<i>Elphidium gunteri</i> Cole	C	C	C
* <i>Elphidium incertum</i> (Williamson)	R	C	
* <i>Elphidium poeyanum</i> (d'Orbigny)	C	C	C
* <i>Nonion grateloupi</i> (d'Orbigny)	C	C	R
* <i>Angulogerina occidentalis</i> (Cushman)		R	
<i>Bolivina beaufortana</i> n. sp.	R	R	

	Station		
	1	2	3
* <i>Reusella spinulosa</i> (Reuss).....		R	
* <i>Discorbis subaraucana</i> Cushman.....	C	C	
* <i>Eponides mansfieldi</i> Cushman.....	R	R	
* <i>Rotalia beccarii</i> (Linnaeus) var. <i>parkinsoniana</i> (d'Orbigny).....	R	R	C
<i>Rotalia beccarii</i> (Linnaeus) var. <i>tepida</i> Cushman.....	C	C	R
<i>Globigerinoides</i> cf. <i>sacculifera</i> (H. B. Brady).....	C	C	
* <i>Orbulina universa</i> d'Orbigny.....	R		
* <i>Globorotalia menardii</i> (d'Orbigny).....		R	
* <i>Cibicides concentricus</i> (Cushman).....	C	C	C
* <i>Dycibicides biserialis</i> Cushman and Valentine.....		R	

Frequency: C—common; R—rare.

Species marked (*) have been reported from beds of Miocene age.

Time range of species: Of the 23 species listed above, 16 occur in beds of Miocene age and younger along the Atlantic coastal area adjacent to the region where they are now living.

Genus BOLIVINA d'Orbigny, 1839

Bolivina beaufortana new species

Small, elongate, moderately compressed; periphery rounded; initial end blunt, test widening but slightly toward the apertural end, sides almost parallel; three or four pairs of chambers distinctly visible and making up most of the test; individual chambers subspherical, slightly inflated and separated by depressed sutures; each chamber decorated with about nine delicate, sharp, longitudinal costae which are limited to the individual chambers and develop into spinose projections at their lower extreme; the greatest concentration of spines is on the early portion of the test; wall thin, calcareous and hyaline; aperture an elongate slit lying in the plane of compression, surrounded by a slightly elevated lip and extending from the apex to the base of the final chamber.

Length—.40 mm.; greatest width—.15 mm.; thickness—.11 mm.

Holotype is from just west of sea buoy off Beaufort Inlet; depth of water—40 ft. Rare at Stations 1 and 2. A topotype of this species has been deposited in the Department of Geology, University of North Carolina, Chapel Hill, N. C.

Bolivina beaufortana differs from *Bolivina pulchella* (d'Orbigny) of Cushman (Carnegie Inst. Washington Publ. No. 311: 25, pl. 1, figs. 8, 9, 1922) in being of nearly uniform width throughout, that is not showing a steady increase in width toward the final end, and by not having the distinct angular development of the chambers at the periphery. *Bolivina pulchella* (d'Orbigny) var. *primitiva* Cushman (Florida

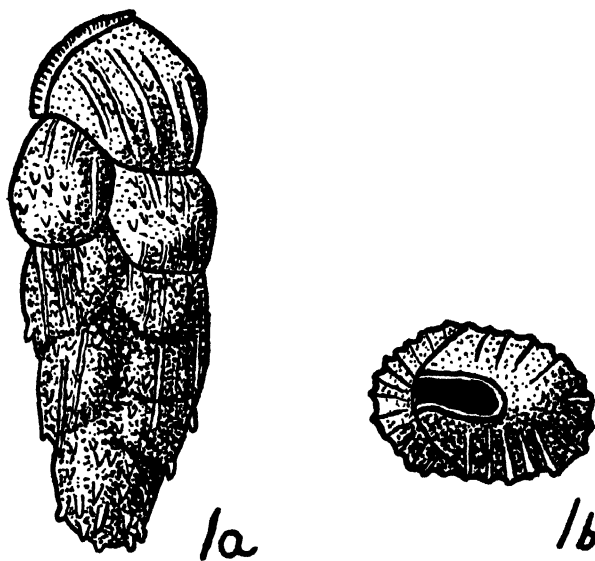


FIG. 1

St. Geol. Survey Bull. 4:47, pl. 8, figs. 12 a, b. 1930) differs from *Bolivina beaufortana* in being practically free of spines.

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THE EFFECTS OF ENDOCRINES ON THE DEVELOPMENTAL RATES OF FLESH FLIES

By E. C. HESTER AND BERT CUNNINGHAM

Anatomical and embryological studies of invertebrates fail to show any organs, with the possible exception of the sex glands, of an endocrinal nature. If, therefore, there is any hormonal substance produced in invertebrates it must be secreted by tissues which are not recognized histologically as differentiated for this purpose.

The fact that endocrine organs as such are not to be found in invertebrates has led many to assume that endocrines would have little or no effect, as indicated by the usual physiological action, when applied to the invertebrates. It was to test this hypothesis that the following experiments were devised and conducted.

Because of their well known effects in vertebrates and because of the ease of procuring fresh glandular material, the thyroid and anterior pituitary were chosen as sources of the endocrines to be tested. Both of these organs produce substances related to growth, metamorphosis, and sexual maturity and should therefore produce significant changes in these processes if they have any effect, whatsoever, on invertebrates. Furthermore it has been shown for vertebrates that thyroid, when administered by feeding, produces as definite physiological effects as when the hormone is injected, although it takes a larger dosage to produce comparable results. While evidence that oral administration of pituitary is effectual is not so extensive, there are a number of experiments reported in the literature where oral administration has been effective.

Flesh flies appear to meet most fully the requirements for the invertebrate experimental animals since they are carnivorous both as larvae and adults. That they may thrive well on endocrine gland substance is evidenced by the fact that stock colonies are commercially raised on fresh thymus. They furthermore have the advantage of having short life cycles (about fifteen days) which may be further shortened or lengthened by controlling the temperature. *Phormia regina* was selected as the test animal.

The stock colony of *P. regina* was secured from the Lederle Laboratories, frozen thyroids from Swift and Co., anterior pituitaries from the

Wilson Laboratories, and ground meat (two-thirds beef, one-third pork) from local markets. Some of the studies were made at room temperatures which were more or less variable, ranging from 22° to 30°C., while others were made at temperatures which were controlled at or below 20°C. by refrigeration. The humidity was not kept constant but all the animals in any series were exposed to the same atmospheric conditions.

The flies were allowed to oviposit on flesh and when the eggs hatched the larvae were transferred to the beef or glandular tissue. When pupation had taken place the puparia were transferred to new containers where they were kept until the adults emerged. Upon emergence the adults were fed beef or gland, sugar and water, and were given opportunity, daily, to lay eggs upon fresh meat.

In this study three fundamental questions have been considered: (1) What effect, if any, does thyroid feeding have on the rate of development (a) at ordinary temperatures, and (b) at reduced temperatures? (2) Is there any cumulative effect transmitted from one generation to another? (3) What effect, if any, does anterior pituitary feeding have on the rate of development?

The data bearing on these points are assembled in Tables 1-5. We shall not attempt to make a complete analysis of the data, but we wish to point out what we think to be some of the more significant findings.

Feeding thyroid at room temperatures seemed to have little if any effect upon the time spent in the larval or pupal stage (see Table 2). The results may be summarized as follows:

	LARVAL LIFE				PUPAL LIFE					
	4 days		5 days		4 days		5 days		6 days	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Beef ..	872	56	667	43	718	54	524	40	69	5
Thyroid .	882	55	719	44	734	55	562	42	37	2 5

These results do not agree with the findings of Kunkel (2), but he was working with another species. Nor do they fully agree with the findings of Brannon (1), who reported that the pupal life was shortened. This difference from our work may be due to the extremely low temperature (10°C.) which he maintained throughout his experiment. Our results do agree with Brannon in that we found no appreciable

TABLE 1
The effects of feeding thyroid on the length of larval life in days

SERIES	MEDIUM	NUMBER OF ANIMALS USED IN EACH SERIES	6 DAYS		7 DAYS		8 DAYS		9 DAYS		TOTAL NUMBER FUPATING	
			Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
I	Beef	100	35	35	58	58	5	5	98	98
I	Thyroid	400	46	11	239	60	8	.2	293	71
II	Beef	100	21	21	6	6	27	27
II	Thyroid	100	7	7	15	15	6	6	28	28
III	Beef	100	78	78	10	10	88	88
III	Thyroid	500	182	32	162	32	12	12	379	76
IV	Beef	100	27	27	54	54	93	93
IV	Thyroid	300	108	36	155	51	263	87
V	Beef	100	48	48	43	43	91	91
V	Thyroid	1,000	14	14	734	73	28	2.8	776	76
VI	Beef	100	31	31	13	13	43	43
VI	Thyroid	400	28	7	197	49	225	56
VII	Beef	100	9	9	38	38	47	47
VII	Thyroid	500	8	2	271	64	279	55
VIII	Beef	100	20	20	50	50	12	12	82	82
VIII	Thyroid	500	318	64	76	15	394	79
IX	Beef	100	20	20	3	3	23	23
IX	Thyroid	500	96	19	51	10	147	29
Total beef.....		900	200	22	264	29	86	9	43	5	593	66
Total thyroid.....		4,200	569	13	1,845	43	245	7	161	5	2,820	67
Grand total.....		5,100	769		2,109		331		204		3,413	

The effects of feeding thyroid on the length of pupal life in days

SERIES	MEDIUM	4 DAYS		5 DAYS		6 DAYS		7 DAYS		8 DAYS		TOTAL NUMBER OF PUPAE EMERGING		NUMBER OF LARVAE BECAME ADULTS	AGE	
		Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent	Num-ber	Per-cent		4	5
I	Beef	..				5	5	70	70	1	1	76	76	77		X
I	Thyroid	2	8	10	37		8	196	67	4	13	271	76	67	X	X
II	Beef	..		1	3	7	25	5	17	..		12	47	12	X	
II	Thyroid											13	46	13		
III	Beef													.		
III	Thyroid															
IV	Beef	..				4	4	63	70	13	14	80	89	80	X	X
IV	Thyroid	10	4	49	19	14	7	8	3	4	1	85	34	28	X	X
V	Beef	25	26	4	4							29	30	29	X	X
V	Thyroid					15	2	333	42	340	45	688	89	69	X	X
VI	Beef			10	23	1	2	2	5	1	2	14	34	14	X	X
VI	Thyroid			1	4	33	10	45	20	44	20	123	40	30	X	X
VII	Beef	2	7	11	23	17	36	3	6			31	59	31	X	X
VII	Thyroid			2	7	33	12	65	23	49	14	151	48	30	X	X
VIII	Beef					4	5	63	76	13	16	80	97	80	X	X
VIII	Thyroid					37	10	303	80	24	6	364	96	73	X	X
IX	Beef			5	22	5	22	7	30			17	74	17	X	X
IX	Thyroid			24	16	17	11	9	6			50	34	10	X	X
Total beef		27	45	40	7	36	6	205	34	29	5	339	55			
Total thyroid		12	42	77	24	177	6	959	34	465	16	1,690	60			
Grand total		39		117		213		1,164		494		2,029				

The nine series represent nine completely separate and independent experiments. Each series consisted of a beef control and one or more groups of thyroid fed animals. Each group within the series consisted of 100 newly hatched larvae which were transferred either to the beef or thyroid. Usually more than one group of thyroid fed animal was placed in a series. The larval life in days is the time intervening between hatching and pupation. The pupal life in days is the time transpiring between pupation and emergence. The column "Age" indicates the time required in days after emergence before oviposition began. Temperature 20°C.

TABLE 2

The effects of feeding thyroid to successive generations, at room temperatures, on the length of larval and pupal life

GENERATION	MEDIUM	LARVAL LIFE						PUPAL LIFE						PER CENT OF LARVAE THAT BECAME ADULTS	AGE		
		4 days		5 days		Total		4 days		5 days		6 days			Total		
		Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent	Num- ber	Per cent		Num- ber	Per cent	
I	Beef	19	19	38	38	57	57	15	15	30	30	45	45	79	X
I	Thyroid	10	10	40	40	50	50	14	14	32	32	46	46	82	X
II	Beef	21	21	50	50	71	71	4	4	63	63	13	13	80	80	88	X
II	Thyroid	76	76	24	24	100	100	2	2	98	98	100	X
III	Beef	68	68	24	24	92	92	30	30	53	53	88	88	95	X
III	Thyroid	65	65	30	30	95	95	16	16	61	61	77	77	79	X
IV	Beef	28	28	7	7	35	35	5	5	20	20	4	4	29	29	83	X
IV	Thyroid	24	24	7	7	31	31	12	12	8	8	3	3	23	23	74	X
V	Beef	30	30	62	62	92	92	5	5	70	70	75	75	81	X
V	Thyroid	34	34	60	60	94	94	6	6	61	61	67	67	71	X
VI	Beef	76	76	12	12	88	88	40	40	15	15	6	6	61	61	69	X
VI	Thyroid	36	36	52	52	88	88	20	20	35	35	7	7	62	62	67	X
VII	Beef	40	40	43	43	83	83	24	24	5	5	29	29	35	X
VII	Thyroid	38	38	30	30	68	68	20	20	25	25	4	4	49	49	72	X
VIII	Beef	30	30	12	12	42	42	10	10	1	1	3	3	14	14	33	X
VIII	Thyroid	8	8	54	54	62	62	31	31	16	16	47	47	76	X
IX	Beef	27	27	62	62	89	89	3	3	60	60	13	13	76	76	85	X
IX	Thyroid	14	14	30	30	44	44	2	2	13	13	17	17	32	32	73	X
X	Beef	31	31	12	12	43	43	18	18	12	12	30	30	70	X
X	Thyroid	30	30	13	13	43	43	5	5	28	28	33	33	76	X
XI	Beef	42	42	4	4	46	46	38	38	8	8	46	46	100	X
XI	Thyroid	28	28	30	30	58	58	37	37	18	18	2	2	57	57	98	X

[illegible]

effect on the length of larval life at room temperatures. It is to be remembered that Brannon used a different species from that which Kunkel used, and the one which we used. In our experiments the time between emergence and oviposition was consistently shortened.

Thyroid feeding at a controlled temperature of 20°C. increased the length of time, as compared to meat, spent in both the larval and pupal forms (see Table 1). The hastening of sexual maturity was also evident under these conditions.

That the thyroid tissue serves as a good basic food for these flies was evidenced by the comparative number of larvae pupating, the number

TABLE 3

The effects of feeding thyroid on oviposition in successive generations

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Number of thyroid adults depositing eggs.....	23	47	40	11	16	9	16	38	8	9	13	16	20	20	30	16	16	22	17	6	8	0	0
Number of thyroid adults depositing eggs after beef feeding.....																					23	19	0
Number of beef adults laying eggs..	18	40	32	13	50	36	6	3	20	7	16	21	40	23	30	16	32	20	11	26	32	16	19
Per cent of thyroid adults depositing eggs.....	50	47	52	49	23	60	31	17	25	27	30	30	40	30	60	22	21	20	22	12	16	30	0
Per cent of beef adults depositing eggs.....	40	50	40	49	66	43	21	22	25	23	39	50	47	29	45	40	45	44	24	40	40	51	30

of pupae emerging and the number of adults laying eggs (see Tables 1 and 2). The failure of thyroid to affect the pupa or larva at room temperatures, and its later striking effect on the adult is disconcerting.

The data relative to the cumulative effects are shown in Table 2. Through twenty-three generations there was no appreciable change, as compared to controls, in the length of time spent as larvae or pupae. While flies produced from thyroid-fed larvae in each generation up to the 21st, became sexually mature earlier than the controls, there was no evidence of any cumulative effect in further shortening the period.

Although there seems to be no relation of the thyroid feeding to the number of egg masses produced in the earlier generations, some influence

TABLE 4

The effects of feeding anterior pituitary at temperatures from 11°C. to 30°C. on the length of larval life in days

SERIES	MEDIUM	TEMPER- ATURE	12	13	14	15	16	17	18	19	20	21	22	23	24
1	Beef	20	14	...	14	...	3
1	Pituitary	20	15	2	5
2	Beef	17	1	15	5
2	Pituitary A	17	13	10	5
2	Pituitary B	17	15	15
3	Beef	15	3	1	5	4	3
3	Pituitary A	15	3	3	7
3	Pituitary B	15	3	8	7	3
4	Beef	13	...	2	3	3	11	...	10
4	Pituitary A	13	1	...	4	11
4	Pituitary B	13	...	3	13
4	Pituitary C	13	7	3	...	8	1
4	Pituitary D	13	...	1	...	2	13
4	Pituitary E	13	4	...	10
5	Beef	13	1	3	1	2	1	2	20	...
5	Pituitary A	13	1	5	2	7
5	Pituitary B	13	4	9	10	1	1	2
5	Pituitary C	13	5	2	1	12	5
5	Pituitary D	13	10	10	6	4	3
5	Pituitary E	13	2	2	...	2	4	15
5	Pituitary F	13	1	...	1	13	10	3
5	Pituitary G	13	3	14	6	2
5	Pituitary H	13	2	...	2	10	12
6	Beef	16
6	Pituitary A	...	5	3
6	Pituitary B	2	...	7	9	17
6	Pituitary C	10	4	...	3
7	Beef	15	1	1	1	2	4	4	3	8
7	Pituitary A	15	6	7	6	...	4	4	1
7	Pituitary B	15	4	6	6	...	3	4	1
7	Pituitary C	15	1	4	7	6	...	2	1
8	Beef	16	3	6	11	7	9	9
8	Pituitary A	16	...	1	15	5	10	3
8	Pituitary B	16	4	18	3
8	Pituitary C	16	2	4	1
8	Pituitary D	16	2	10	13
8	Pituitary E	16	...	1	17	11
9	Beef	13	1	10	12
9	Pituitary A	13	8	4	...	16	1
9	Pituitary B	13	1	6	5	...	3
9	Pituitary C	13	1	3	...	6

TABLE 4—Continued

SERIES	MEDIUM	TEMPER- ATURE	12	13	14	15	16	17	18	19	20	21	22	23	24
9	Pituitary D	13			1			1			1				
9	Pituitary E	13		6	12	6		6	7						
10	Beef	14									9	4		3	
10	Pituitary	14						2	6	15	12	3		1	
11	Beef	14								1	4	4	2	8	8
11	Pituitary A	14										5	1	10	
11	Pituitary B	14							2	9	12				
11	Pituitary C	14											5	2	
11	Pituitary D	14						5	6	12	2				
11	Pituitary E	14							2	6		2	3	2	
11	Pituitary F	14									2	6	5		
11	Pituitary G	14						1	7	3	11				
11	Pituitary H	14									4	10	9	5	

The effects of feeding anterior pituitary at temperatures from 11°C. to 20°C. on the length of pupal life in days

SERIES	MEDIUM	TEMPER- ATURE	13	14	15	16	17	18	19	20	21	22	23	24	25
1	Beef	20					11								
1	Pituitary	20		3	7	6	3								
2	Beef	11												13	
2	Pituitary A	11							4	10	5	2	1		
2	Pituitary B	11									4	5	16	6	
3	Beef												2	10	
3	Pituitary A									4		5	3		
3	Pituitary B									2	2	5			
4	Beef	13							2	2	13	13			
4	Pituitary A	13								3	9				
4	Pituitary B	13					1			8	6				
4	Pituitary C	13								1	5				
4	Pituitary D	13	..							5	4	2			
4	Pituitary E	13	...				4				1				
5	Beef				1	2	1		2	5	2		
5	Pituitary A						8	10	3			..	
5	Pituitary B				2	5	12	2		2	..		
5	Pituitary C							1	7	3			
5	Pituitary D						12	5	3						
5	Pituitary E							1	3	6	3	7			
5	Pituitary F									1	2	15			
5	Pituitary G			...						10	1		2		

TABLE 4—*Concluded*

SERIES	MEDIUM	TEMPERATURE	13	14	15	16	17	18	19	20	21	22	23	24	25
5	Pituitary H								2	1	5	10			..
6	Beef					12									
6	Pituitary A				5	1									
6	Pituitary B		14			9									
6	Pituitary C			7	2	2									
7	Beef									3		2	2	2	3
7	Pituitary A									15	2	4	2		
7	Pituitary B									5	1	5	3		
7	Pituitary C										1	2	2	3	
8	Beef									13	11		5		
8	Pituitary A								3	3	11				
8	Pituitary B								6	12					
8	Pituitary C								1	12	1	5			
9	Beef	11													15
9	Pituitary A	11											9	9	1
9	Pituitary B	11											2	1	8
9	Pituitary C	11											2	1	3
9	Pituitary D	11									9	4	3		
9	Pituitary E	11									10	4	6		
10	Beef												1	1	5
10	Pituitary									3	8	8	2		
11	Beef				1		5			5					
11	Pituitary A						1	2	4						
11	Pituitary B				9		14								
11	Pituitary C						5	7		1					
11	Pituitary D					10	6	4							
11	Pituitary E						4	3							
11	Pituitary F				1		6	2	1						
11	Pituitary G				15	7	2	2	3						
11	Pituitary H				1		11	1							

These experiments were set up in the same manner as those shown in table 1. Each series consisted of a control (beef fed) and one or more groups of pituitary fed animals. Each group at its beginning consisted of 100 newly hatched larvae. Where the temperature remained fairly constant during the experiments they have been set down, but where they were variable to any great degree the temperatures have been omitted from the table. In such cases however the temperature rarely rose above 20°C.

seems to be evident after the 15th generation (see Table 3). This condition became critical in the 21st generation where there was a marked reduction in the number of "thyroid-fed flies" that oviposited. When, however, these flies were allowed to feed on beef many of them deposited

The effects of feeding anterior pituitary were studied only at lowered

temperatures, ranging from 11° to 20°C., where rather striking results were secured. The larval life was shortened as was also the pupal life (see Table 4). The puparia formed were also larger than the controls (see Table 5). These results do not agree with those of Patterson (3) who, working with a larviparous flesh fly, was unable to find any effect of pituitary on the growth of the larvae or the size of puparia. His experiments involved at least 20 different set-ups including controls, and less than 200 individuals were considered in the entire study. Furthermore he used "slightly decomposing" pituitary substances as media.

Since the time required by our experimental animal for reaching sexual maturity after emergence did not seem to be appreciably different from the controls, the data have not been tabulated here.

The growth effects of pituitary feeding are not surprising since a growth factor is thought to be present in the pituitary. In this case however, the hormone must work directly upon the tissues. The failure of pituitary feeding to hasten sexual maturity is surprising since the gland normally contains a gonad stimulating hormone, and it was shown herein that the *Phormia regina* gonad can be stimulated by thyroid feeding.

SUMMARY

At room temperatures the length of larval and pupal life were not modified by thyroid feeding as compared to beef feeding, but the time between emergence and oviposition was shortened.

At a temperature of 20°C. both larval and pupal life was lengthened as compared to the controls. Under these conditions also the time required for reaching sexual maturity was shortened.

At temperatures ranging from 11° to 20°C. pituitary feeding shortened the length of both larval and pupal life as compared to controls. However no difference as to the time required to reach sexual maturity after emergence was observed between pituitary and beef fed animals.

There were no cumulative effects observed during some twenty-three generations but sterility appeared rather suddenly in the 21st and 22nd generations of the thyroid fed animals.

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THE PHOTOCHEMICAL DECOMPOSITION OF CARBON DISULFIDE

By P. M. NICHOLS, N. L. SIMMONS, AND H. D. CROCKFORD

O. Loew (5) states that when carbon disulfide is exposed to sunlight a deposit of carbon trihemisulfide, C_2S_3 , is formed on the walls of the container. Th. Sidot (7) states that carbon monosulfide, CS , is formed upon exposure to sunlight. The product is a red powder of approximately 1.65 specific gravity. Dewar and Jones (2) considered the product of Sidot to be a polymer of the monosulfide. Doran and Gilliam (3) studied the photochemical decomposition of carbon disulfide in carbon tetrachloride solution and found a product with properties similar to the above. They considered the product to be $(CS)_x$.

The absorption spectrum of carbon disulfide in the ultra violet has been studied by Bruhat and Pauthenier (1), Wilson (8), and Haverlock (4). From a consideration of the work of these investigators it can be concluded that carbon disulfide absorbs all wave lengths between 1900 and 3600 Å with the exception of a short range between 2700 and 2900 Å. The maximum absorption is found at approximately 3200 Å.

In this paper are given the results of a study of the decomposition of liquid carbon disulfide upon exposure to ultra violet light. Three sulfides of carbon were obtained besides elemental sulfur. These three sulfides were: a red-brown powder of probable formula $(CS)_x$, a red liquid of formula C_3S_2 , and a brown solid which is probably a polymer of the latter.

METHODS OF ILLUMINATION

Mercury arc lamps made of Pyrex glass and cooled by water jackets were used as the principal sources of illumination. The use of Pyrex glass of course fixed the short wave decomposition limit at 2900 Å. The carbon disulfide was contained in Pyrex tubes of approximately three inches diameter. These were either sealed off or closed with tight-fitting stoppers. Experiments proved that small quantities of air in the tubes had no effect on the results. Tests were also made with a quartz mercury vapor lamp emitting monochromatic radiation of 2537 Å.

The carbon disulfide in these cases was contained in quartz vessels. Similar results were obtained with both types of lamps.

In order to test the long wave length decomposition limit certain samples were illuminated with light filtered through a Corning No. 586 glass filter. This, in conjunction with the lamp used, produced light of approximately 3660 Å wave length. Other samples were illuminated in tubes made of soft glass. In both cases little, if any, decomposition took place. These experiments fix the long wave length limit at approximately 3600 Å which is in agreement with the conclusions drawn from the absorption spectra data.

It is thus seen that the results given in this paper are due to the action of light between the limits of 2900 and 3600 Å.

PRODUCTS OBTAINED

As stated the products obtained were as follows: a red-brown precipitate of probable formula $(CS)_x$; a dark red liquid, carbon ditritasulfide, C_3S_2 ; a brownish-yellow film which is probably a polymer of the ditritasulfide; and free sulfur. These products were formed in very small quantities so that microchemical methods of analysis had to be used, in most cases the individual samples being approximately ten milligrams in weight. The analyses are accurate to ± 1 percent.

The first appearance of solid material when carbon disulfide was exposed to the radiations was the formation of minute particles, almost colloidal in nature, which upon standing for some hours after illumination formed a red-brown precipitate. This was recovered from the liquid by filtration through crucibles with sintered glass bottoms. The precipitate was carefully washed with hot carbon disulfide, ether, and acetone. It was tasteless; odorless; apparently insoluble in the ordinary organic solvents, dilute sulphuric acid, dilute nitric acid, and sodium hydroxide solution. It was slightly soluble in cold, concentrated nitric acid and dissolved readily in the hot acid to produce a red solution. It dissolved in concentrated sulfuric acid, the solution changing to a purplish-brown color when heated. Dilution with water in both cases produced a reprecipitation. The substance was somewhat soluble in pyridene but no suitable solvent was found for purposes of purification. An attempt to obtain the melting point was unsuccessful as decomposition with the formation of carbon and sulfur took place over a range from 220 to 240°C.

The powder was analyzed for sulfur and carbon according to the methods given by Pregl, "Quantitative Microanalysis." For accurate

details the reader is referred to this book. Briefly the process for sulfur was as follows. The sample, previously dried for several days at 125° , was weighed into a porcelain boat which was placed in a combustion tube, approximately 50 cm. in length, one end of which was filled for a distance of 20 cm. with glass beads, the surfaces of which were covered with 30% hydrogen peroxide solution. Between the beads and the sample were located two pieces of platinum foil catalyst. Carefully purified oxygen was passed over the sample with proper heating. The resulting sulfur dioxide was catalyzed to sulfur trioxide by the platinum catalyst. This trioxide was in turn converted by the hydrogen peroxide to sulfo-mono-peracid, H_2SO_5 , which after removal was titrated, methyl orange being employed as the indicator. For the carbon a quartz combustion tube was used, the resulting carbon dioxide being absorbed in soda lime after the oxides of sulfur were removed by bubbling through chromic acid. Combination of the sulfur and carbon analyses were unsuccessful. It was found in both cases that complete combustion was impossible, a residue, making up about 15% of the total, remaining after combustion. The part which reacted gave for carbon 26.3% and for sulfur 73.7%. It is seen that this analysis corresponds closely to the composition CS , which formula gives a theoretical sulfur value of 72.7%. This would indicate an incomplete removal of the free sulfur from the powder.

As the carbon monosulfide described by Mellor (6) is a gas in the unpolymerized state the product obtained by us is undoubtedly a polymer. As described by Mellor such a polymer has been prepared by several investigators, the properties being very similar to those of the powder. For purposes of comparison $(\text{CS})_x$ was prepared by us according to the method of Dewar and Jones (2). This method employs the reaction between thiophosgene and nickel carbonyl. The product so obtained was identical with the brown powder both in respect to properties and analysis. The same residue was found on combustion.

The red liquid was soluble in carbon disulfide and was obtained by evaporating the filtrate from the solid products under reduced pressure. The resulting dark red liquid contained some dissolved sulfur which was separated by dissolving the red liquid in ethyl alcohol. However this method is only partially successful. To prevent polymerization the liquid was stored in acetone from which it was recovered by evaporation of the solvent. The liquid was analyzed for carbon and sulfur according to the methods employed for the $(\text{CS})_x$. The analysis

showed: carbon, 34%; sulfur, 66%. The liquid is characterized by a strong, penetrating odor and low vapor pressure. It is soluble in most organic solvents imparting to the solutions a red-brown color. When heated in a small test tube a black mass results with the formation of free sulfur and the evolution of a gas with a mercaptan-like odor. An attempt to freeze the liquid was unsuccessful. It became very viscous and although temperatures as low as $-20^{\circ}\text{C}.$ were employed complete solidification was never obtained. The liquid shows a decided tendency to polymerize in the free state and in carbon disulfide solutions. A semi-hard black mass results. Moreover on the sides of the container a film is formed similar in appearance to that found in the original containers employed by us for exposing the carbon disulfide to the radiations.

The properties agree very well with those of carbon ditritasulfide, C_3S_2 , described by Mellor (6). This has been prepared and studied by several investigators as stated in the above reference. However, while our product had a penetrating, disagreeable odor it did not have the destructive and harmful effect on the mucous membranes of the nose as stated by Mellor. Nor were we able to secure a definite melting point. Mellor states that solidification should take place at $-.5^{\circ}\text{C}.$ The sulfur content of carbon ditritasulfide is 64%. The sulfur content of the red liquid is somewhat higher than this. In fact the analysis conforms more closely to C_4S_3 rather than C_3S_2 . However due to the difficulty of removing the last traces of free sulfur a high sulfur percentage is not hard to explain.

After about eight hours exposure a brown film formed on the side of the reaction vessel nearest the source of illumination. By turning the tube a uniform deposit could be obtained. Once formed this film served to effectively prevent further penetration of the light. The film deposited was easily displaced by the addition of water with subsequent shaking. The material was in the form of thin, semitransparent flakes of a brownish-yellow color. The method of separation from the liquid was essentially the same as that employed for the red powder. It was found impossible to secure this film free of the powder. The final product appeared amorphous under the microscope. There seemed to be no true melting point, decomposition taking place slightly above $200^{\circ}\text{C}.$ with the formation of carbon and sulfur. From the standpoint of solubility the properties were similar to those of the red-brown powder, $(\text{CS})_x$. The same action was found with the inorganic acids. Analysis for carbon and sulfur in the usual way showed 31.5% car-

bon and 68.5% sulfur. In view of this analysis and the great similarity of this film to the product obtained from polymerization of the C_3S_2 it was concluded that this film was a polymer of the carbon ditritasulfide. The high sulfur percentage was no doubt due to the fact that the film could not be completely purified from the brown powder. As with the $(CS)_x$ complete combustion could not be secured, a residue always remaining. It should be added that there seems to be no record in the chemical literature of a polymer of C_3S_2 of this type.

SUMMARY

1. The photochemical decomposition of carbon disulfide has been studied using the full radiation from quartz and mercury vapor lamps.
2. Besides elemental sulfur three sulfides of carbon have been obtained as products: a red powder, $(CS)_x$; a red liquid, C_3S_2 ; and a yellowish-brown solid, $(C_3S_2)_x$.

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THE STRUCTURE AND DEVELOPMENT OF THE SEED OF PAULOWNIA TOMENTOSA STEUD.*

By VERA MILLSAPS

PLATES 4-6

HISTORICAL INTRODUCTION

The ovules of the Scrophulariaceae have been studied by various workers since the time of Hofmeister. In his classic work of 1849 Hofmeister (11) stated on the basis of his examination of a large number of species that the embryo developed from a fertilized egg, and expressed the belief that this phenomenon was common in all phanerogams. It is interesting to note that he used two genera of the Scrophulariaceae, *Lathraea squamaria* and *Pedicularis sylvatica* for further investigation of this subject in 1851, and proved unmistakably that the embryo was formed as the result of a fertilized egg (12). These facts undermined the pollen-tube theory of Schleiden who had maintained in 1835 that the embryo developed from the tip of the pollen tube (8, 18). Hofmeister's researches, published in 1855, were so decisive that they discredited Schleiden's contention for all time (13).

Pedicularis sylvatica was the subject of considerable controversy in 1855 between Deecke (9) and Schacht (17) on the one hand and Hofmeister (13), von Mohl (16), and Tulasne (23) on the other. Deecke reinvestigated *Pedicularis* in 1855 and claimed that Hofmeister was wrong in saying that the embryo did not develop from the tip of the pollen tube (9). Schacht agreed with Deecke's statements. Hofmeister cleared up the whole matter by showing that what Deecke had really seen was the proembryo (13).

The next work of any importance on the Scrophulariaceae was done by Chatin in 1874 when he investigated the development of the ovule and the seed in several genera, putting special emphasis upon *Veronica* (5). In 1879 Vesque investigated the embryo sac in a number of families, including several members of the Scrophulariaceae (25). Even

* Part I of a thesis submitted to the Faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Botany.

at this time, twenty-four years after Hofmeister's decisive statement concerning embryo formation, Vesque still believed that Schleiden's theory was correct.

Three years later, in 1882, Bachmann (1) made an intensive study of the structure and development of the seed coat of a large and representative number of the Scrophulariaceae. He found three layers in the testa of the majority of species examined, the outer one usually being strengthened by a characteristic net thickening.

In 1899, Balicka-Iwanowski made a study of the embryo sac in a number of the Sympetalae, dealing especially with several genera of the Scrophulariaceae (2). This paper remains a classic in its field. This worker emphasized the question of nutrition in the embryo sac and believed that the haustoria could absorb food and conduct it to the embryo sac. She did not consider the tapetum as a protective covering, but as a layer of cells having a digestive and nutritive function.

In 1906, Schmid made a valuable contribution to our knowledge with reference to the Scrophulariaceae (20). He confined himself entirely to this family, discussing the formation of the embryo sac, fertilization, endosperm formation, and the development of the haustoria. He paid special attention to the development of the endosperm and arranged the genera which he studied into four groups according to their method of endosperm formation.

Verbascum, *Scrophularia*, and *Digitalis*, of his first group, form four superimposed primary endosperm cells of which the outer two form feeble haustoria while the inner two function as true endosperm.

The first endosperm cell in the members of the second group, consisting of *Linaria* and *Antirrhinum*, divides transversely. The micropylar cell thus formed develops the endosperm and a few of the endosperm cells at the micropylar end function as haustoria. The lower cell forms a tube-like haustorium in which the nucleus divides once though no cell wall is formed.

Alectorolophus and *Lathraea* form the third group. Here again the lower cell forms a haustorium. The micropylar haustoria are formed by two cells which arise from the micropylar end of the upper endosperm cells and become binucleate.

The fourth group, consisting of *Veronica*, *Euphrasia*, *Pedicularis*, *Melampyrum*, and *Tozzia*, is like the third except that the micropylar haustorium originates from one cell and has four nuclei.

It is Schmid's opinion that the endosperm characters should be considered in making a natural classification of the family.

A study of the genus *Pentstemon* was made by Krautter in 1908, in which he paid considerable attention to a comparative study of the seeds of the genus with regard to weight, size, number and structure of layers of the testa, and external appearance (14).

The next definite work was done on the Scrophulariaceae in 1915 by Margaret Michell who investigated the embryo sac and embryo of *Striga lutea*, a semi-parasitic member of this family which is common on maize in South Africa (16). In this species the endosperm is formed by cell division. A long binucleate haustorium penetrates the chalazal region while a few endosperm cells function as an inconspicuous micropylar haustorium. The proembryo has a long suspensor of three or four cells, the basal one of which forms tuberosus haustoria. According to Miss Michell this is the only case of such haustoria recorded for the Scrophulariaceae. There is no definite tapetal layer.

Four years later, in 1919, Arthur T. Evans studied the embryo sac and embryo of *Pentstemon secundiflorus* (10). The 8-nucleate embryo sac in this species is decidedly club-shaped, much more so than any other known in the family. The chalazal end is usually long and narrow, and is surrounded by a tapetum. The endosperm nucleus divides immediately after its formation and free nuclei move down into the chalazal end, a narrow neck being left between. Wall formation begins and continues until the space is filled with cells. The proembryo is pushed down into this endosperm by unusual growth of the suspensor. Four endosperm cells which become binucleate form the chalazal haustorium. Two endosperm cells form the micropylar haustorium by pushing up through the narrow neck into the micropylar end of the sac. Their growth stops as soon as the embryo is pushed down between these cells into the endosperm. The whole micropylar region of the sac finally disappears due to the pressure of the increasing endosperm.

In the same year, 1919, F. M. Schertz investigated the embryo sac of *Scrophularia marylandica* (19). In this species the tapetal layer completely surrounds the embryo sac. Endosperm is formed by cell division, and the embryo sac is filled with cells before the egg divides. There are two well developed haustoria in the chalazal region and four small ones at the micropylar end of the sac.

A recent paper by Melville T. Cook on the development of the seed of *Linaria vulgaris* appeared in 1924 (7). In this species also the embryo sac is entirely surrounded by tapetum. The endosperm is of the cellular type, but there are no haustoria developed. Cook states that he found the majority, and in some cases all, of the seeds in an ovary without

embryos. Even in some cases where pollen tubes had entered sacs no embryos were formed. The endosperm develops in the usual way regardless of the presence or absence of an embryo.

The present paper deals with the structure and development of the seed of *Paulownia tomentosa* Steud., a plant belonging to the sub-family Antirrhinoideae of the Scrophulariaceae. A native of China, it was introduced into this country from Japan about 1834 as an ornamental tree. It has since escaped from cultivation and is now sparingly spontaneous from New York southward.

The writer is indebted to Dr. W. C. Coker for his direction and helpful criticism throughout the progress of this work, and to Dr. H. R. Totten for his supervision and kindly aid with details pertaining to the study. Thanks are also extended to Dr. John N. Couch for his constant interest in the problem and many helpful suggestions with regard to techniques, to Miss Alma Holland for valuable advice concerning the preparation of the plates, and to Dr. Velma Matthews for generous help in locating numerous references in the literature.

MATERIAL AND METHODS

The material for this investigation of *Paulownia tomentosa* was collected from trees in Chapel Hill, N. C., during the years 1933 and 1934 except for a small amount that was collected from one tree in Statesville, N. C., during September, 1933. Floral buds begin to form during the first week in August for the next year's flowers at the time when the present year's fruit is maturing; hence collections had to be made throughout an entire year.

The material was killed in medium chromo-acetic solution, formalin acetic alcohol, and medium Flemming's fluid during the early part of the work. Later it was discovered that a combination of Carnoy's and Nawaschin's fixing fluids gave the best results and their use was adopted for the latter part of the work almost entirely. The material was put in Carnoy's fluid for 8-10 minutes and then allowed to stand in Nawaschin's fluid overnight.

Some difficulty was encountered in cutting the early stages of the bud due to the hardening of the tissues during dehydration with ethyl alcohol and xylol. This was overcome by the use of ethyl alcohol and n-butyl alcohol for dehydration (27). These were used entirely in all the later work with all stages.

Haidenhain's iron-alum haematoxylin, a combination of Delafield's

haematoxylin and erythrosin, and Flemming's triple stains were used. The most satisfactory results were obtained with Flemming's triple stain, or with safranin and gentian violet alone, on material that had been fixed with Carnoy's and Nawaschin's fluids. Feulgen's stain was used with good results for staining nuclei in germinated pollen tubes (21).

DEVELOPMENT OF THE MICROSPORANGIUM AND THE MALE GAMETOPHYTE

Floral buds begin to form about the first of August. Sections made from very young buds collected about August 17th show the beginning of the four lobes of the anther and the presence of two layers of cells just within the epidermis that are already differentiated as the primary parietal and primary sporogenous layers. Growth is rapid and a little later sections show two or three rows of parietal cells and several rows of sporogenous tissue. Figures 2 and 3 show a mass of young spore mother cells surrounded by a single row of tapetum.

Four rows of parietal cells is the usual number on the outer curves of the lobes, though there may be five toward the sides of the lobes. The innermost of these layers becomes the tapetum, the cells of which soon become more deeply staining and multinucleate (Fig. 3). The cells of these parietal layers tend to flatten somewhat, so that by the time the anther is mature its walls are much thinner than when first formed (Figs. 7, 16). There is no special differentiation of parietal tissue to facilitate dehiscence except that at the bottom of the groove separating the two lobes there are two thin lines in the wall, where the break occurs.

In sections of anthers collected September 12th practically all later stages were found. There are pollen mother cells well rounded off and separated from each other within pollen sacs completely lined with large binucleate tapetal cells; diad stages of the first reduction division showing late metaphase and telophase stages of nuclear activity; early and mature tetrad stages of microspores, accompanied by slightly less turgid tapetal cells; and also uninucleate spores lying free within the spore cavity. In some flowers uninucleate spores with recognizable thin spots in the exine were found as early as September 4th. By October 17th the tube nucleus and generative nucleus are present, but show no particular difference in size. The tube nucleus is definitely larger by November 9th, and the smaller generative nucleus is more deeply staining and enclosed by a lenticular cell embedded in the protoplasm of the spore. This is the condition when the pollen is shed some time in

April. Pollen grains sprouted in 5% sugar solution soon show the two nuclei in the tube (Fig. 15).

The pollen mother cell stage here occurs more than a month earlier than in *Salix* (3) and *Populus* (4), though in the latter sporogenous cells appear in July, or almost a month earlier than in *Paulownia*. Notwithstanding this early start, the winter is passed in the mother cell stage in those genera. In *Corylus* and *Alnus*, however, also reported by Chamberlain (4), fully formed spores with two nuclei are found in midwinter, as in *Paulownia*. It is probable that most trees and herbs that flower in the early spring develop their microsporangia before the end of the "growing season" and carry them through the winter in the mother cell stage (8). The large flower buds of *Paulownia*, a centimeter or more in diameter, the size being largely due to the very thick, brown, felt-like calyx, occur on terminal racemes in numbers ranging from 50 to 150 or more, and make a very conspicuous appearance throughout the fall and winter months.

DEVELOPMENT OF THE MACROSPORE AND THE EMBRYO SAC

The ovary of *Paulownia tomentosa* is of the bilocular scrophulariaceous type with the partition greatly swollen at the mid-point on the median line and forming in each locule a large almost hemispherical placenta which bears a great number of anatropous ovules. As many as 2000 seeds have been counted in a single average sized capsule (26). Longitudinal sections of the ovary supply a great number of ovules in each section for study.

The ovules begin to arise from the placenta about the same time that the sporogenous layer in the anther becomes well differentiated, forming little protuberances all over the surface. The archesporial cell is recognizable at an early stage before the integument arises by its larger size, larger nucleus, and different reaction to stains, all of which are characteristic of the family (20). This type of cell is found in collections as early as August 20th. It arises from a single hypodermal cell which, without further division, becomes the megaspore mother cell directly. No parietal cell is cut off, thus conforming to the general rule among investigated Sympetalae (8).

The young ovule grows rather rapidly and by September 12th the origin of the integument is discernible. It grows partly up about the single layered nucellus and remains in this condition until activity is resumed in the spring. The nucellus makes a rounded protuberance at the tip of the ovule, which is completely anatropous by the middle of October (Fig. 17).

During the month of March activity again begins in the megaspore mother cell and the nucleus enters synapsis. In the early stages the nucleolus is quite conspicuous but it later disappears. The mothercell is now quite large and its vacuolate, granular protoplasm stains more deeply than the surrounding tissue (Fig. 18). Occasionally an ovule is found which contains more than one megaspore mother cell. One from a collection of March 10th shows three such cells with their nuclei in synapsis (Fig. 19). No further stages in the heterotypic division were observed. Growth is resumed in the integument at the same time that the megaspore mother cell begins to grow as is indicated by its advance upon the nucellus, and the presence of spindles in its cells (Fig. 18). By the time that the two cells produced by the heterotypic division are organized the integument has enclosed the nucellus completely and elongated so much beyond it that these cells now appear toward the base of the ovule. These two cells now divide to form four megaspores and the upper three gradually disintegrate (Figs. 22, 55). Remnants of these are frequently visible as darkly stained masses (Fig. 23).

The development of the embryo sac conforms to the normal type. The nucleus of the functional megaspore divides and the resulting nuclei move one to each end of the sac. The sac is considerably elongated by this time and the protoplasm much vacuolated (Fig. 23). It is usual for the chalazal nucleus to divide a little before the upper so that for a short time the sac is 3-nucleate.

The embryo sac in the 4-nucleate stage is still more elongated, one-half being swollen and flared gradually toward the micropylar end (Fig. 24). The sac at this stage rather closely resembles that of *Digitalis purpurea* (2), and it is not so much unlike that of *Pentstemon secundiflorus* except for the greater elongation of the chalazal end in that species (10). Evans considered this elongation the most striking in the entire family of the Scrophulariaceae. The change from a 1- to a 4-nucleate sac takes place during the time that the corolla is expanding out of the calyx, and the sac apparently remains in this condition for several days, at least until the corolla has fallen off. The flowers begin to open in this locality about the first of April and continue to bloom for about one month.

Almost simultaneously with the appearance of the 8-nucleate stage the polar nuclei, one at each end of the sac, become recognizable by their larger size (Figs. 25, 26). These soon move toward each other until they meet near the middle of the sac. The polar nuclei may fuse

at other places, but they were found so repeatedly just at the entrance to the narrow part of the sac that one is led to conclude that this is the usual place of fusion.

Figure 27 shows an 8-nucleate sac. The polar nuclei are just in position for fusion. The three small antipodals are clear and distinct, and one lies somewhat in advance of the others. This is the usual position of the antipodals, though they are occasionally in a row one above the other (Fig. 28). The egg apparatus consists as usual of two pear-shaped synergids and an egg which is slightly larger than the synergids. The egg has its nucleus in the lower end and a large vacuole in the micropylar end. Its protoplasm seems slightly more dense and granular than that of the synergids, and stains somewhat darker. The vacuoles and nuclei are arranged in just the reverse order in the synergids.

The antipodals usually disappear soon after the complete fusion of the polar nuclei. Often they show signs of disintegration before the polar fusion is completed. This seems to be true in general for the family, though in a few species that have been studied no antipodals were observed. Schmid did not find any in *Melampyrum*, *Antirrhinum majus*, or in *Linaria vulgaris* and *Linaria alpina* (20). Balicka-Iwanowski states that the antipodals are perfectly distinct and persistent in *Linaria Cymbalaria* until the time of the complete formation of the chalazal haustorium (2). Either the behavior of the antipodals varies in the different species of *Linaria* or else Schmid did not have material showing this particular stage. Balicka-Iwanowski further states that none of the Rhinanthae present any very distinct antipodals except *Pedicularis sylvatica*.

The protoplasm of the embryo sac is exceedingly vacuolated, so much so that often only web-like strands which suspend the nuclei in place seem to be left after fixation and staining. There are certainly no starch grains stored here as in the case of *Pentstemon secundiflorus* which Evans reports as having a strikingly large amount, particularly before the time of fertilization (10).

Nucellus

A nucellus consisting of one layer of cells surrounds the megaspore mother cell. Later when growth is resumed in the spring the single massive integument grows up around and far beyond the nucellus, which, with its included megaspore, now lies in the lower part of the ovule (Fig. 55). The cells of the nucellus are long and narrow, par-

ticularly after the cell enclosed by them increases in size, and their transverse walls are usually oblique (Figs. 21, 22). The developing embryo sac breaks through the nucellus at the micropylar end and grows on beyond leaving the nucellus to surround only the narrow chalazal end of the 8-nucleate sac (Fig. 27). The nucellar cells soon collapse, the nuclei disintegrate, and by the time that two to four endosperm nuclei have been formed the nucellus has completely disappeared.

A single-layered nucellus of transitory nature has been reported for *Pentstemon secundiflorus* (10), *Striga lutea* (16), *Scrophularia marylandica* (19), and a persistent one for *Linaria vulgaris* (7).

Tapetal Layer

The tapetal layer appears very early as a band of cells surrounding the nucellus. They arise from the first layer of cells of the integument and begin to be differentiated by their slightly increased size and deeper staining properties as early as the 4-megaspore stage of the embryo sac (Fig. 55). These cells lie with their longitudinal axes perpendicular to the sac. The tapetum, or "nutritive jacket" as it has been termed by Coulter and Chamberlain (8), surrounds the chalazal, or narrowed portion of the sac, almost the same region as that surrounded by the nucellus, and never extends around the expanded portion of the 8-nucleate embryo sac. This layer is more persistent than the nucellus although its content later diminishes and its identity is lost by the time that the embryo sac is well filled out with endosperm cells and the embryo is well advanced. The tapetal cells have large nuclei and contain an abundance of rich protoplasm which stains darker than the surrounding tissue. These facts indicate a nutritive relation to the embryo sac. All of the species of the Scrophulariaceae thus far studied except *striga lutea* (16) show a tapetal layer present though variable in extent in the different kinds. Such a layer perhaps more completely surrounds the embryo sac in *Scrophularia marylandica* than in any other species previously reported (19).

Nutritive Tissue

During the early development of the embryo sac a mass of nutritive tissue develops in the chalazal region of the ovule. The cells of the tissue are small, have small nuclei, and stain conspicuously, indicating that they are different from the surrounding cells (Fig. 55). As the ovule matures its chalazal end pushes out somewhat laterally and the center of this projection is filled with nutritive tissue (Fig. 44). In

older ovules the middle portion of the nutritive tissue stains less than the outer region showing that food has been withdrawn by means of the chalazal haustorium which penetrates it.

In the micropylar region also, at a later period soon after the endosperm has begun to form, a similar mass of nutritive cells, two to three layers thick, develops around the lower part of the micropyle just above the tip of the embryo sac (Figs. 47, 57).

There is no micropylar nutritive tissue in *Digitalis purpurea*, *Linaria Cymbalaria*, and *Torenia Deli*, the last mentioned being almost completely lacking in chalazal nutritive tissue also (2).

FERTILIZATION

Numerous pollen tubes were seen in micropyles and often their swollen tips protruded within the embryo sac cavity (Fig. 33). Tangled masses of them spread out over the surface of the placenta were frequently recognizable. Since the contents of the pollen tube stain rather deeply and uniformly, no nuclei were identified within them. The pollen tube enters the embryo sac somewhat to one side and in so doing injures one of the synergids. The other synergid usually persists until after a few endosperm nuclei are formed (Fig. 33).

Two small and slightly oval sperm nuclei were seen in a number of instances. Many cases were observed where one sperm was in or near the egg. The most difficult stage to find was that of two sperms, a fused polar, and an egg in the same section, but at least four such sections were seen.

Figure 30 shows a pollen tube at the entrance of an embryo sac containing an egg, one synergid partially intact, and a fused polar nucleus. Two small sperm nuclei are visible over the egg. One of them is evidently on its way to the fusion nucleus and the other to the egg nucleus.

Figure 31 also shows two sperm nuclei. The form of the sperms was particularly distinct in this section and showed conclusively that they are somewhat elongate and slightly curved in shape. In this case the fused polar nucleus was cut off in the adjoining section.

Figure 32 illustrates the stage just following fertilization. The nucleus of the egg is large and deeply stained. The big definitive nucleus is still lying close to the egg and contains two prominent nucleoli.

It is evident that there is some variation in the rate of fusion of the egg and the sperm nucleus. In Figure 33 they have not yet fused although division has already taken place in the triple fusion nucleus

and two endosperm nuclei have been formed. The size of the egg nucleus in Figure 32 indicates that fertilization has taken place even though division has not yet occurred in the triple fusion nucleus.

It is certain, therefore, that double fertilization does occur in *Paulownia tomentosa*. In the family of the Scrophulariaceae double fertilization has previously been announced for *Digitalis purpurea*, *Linaria vulgaris*, *Melampyrum sylvaticum*, *Lathraea squamaria*, *Pedicularis foliosa*, *Striga lutea* (15), and *Pentstemon secundiflorus* (10).

The exact length of time between pollination and fertilization was not ascertained, but it is approximately a week to ten days. As has already been noted, sections from marked flowers show that the embryo sac is in the 4-nucleate stage at time of blooming and remains in this stage until the corolla falls off, a period of usually three or four days. The ovary increases rapidly in size during the next few days, the greenish style, which is about three centimeters in length, stands out conspicuously for a time then gradually begins dying from the tip downward so that by the end of a week or more the style has completely withered. Sections of ovules cut at this stage show an abundance of pollen tubes and organized egg apparatuses, large fusion nuclei about the center of the sacs, and numerous cases of sperm nuclei within egg cells. Collections of material were made at all times of the day, killed both at the tree and almost immediately in the laboratory, but no material was collected during the night. It is evident, judging by the large number of slides observed, that actual fertilization must usually take place at night, that the fusion of the sperm nucleus with the fused polar nucleus occurs rapidly, and that the secondary endosperm nucleus travels quickly away after fusion toward the center of the sac.

FORMATION OF ENDOSPERM

Endosperm formation begins soon after fertilization. The endosperm nucleus divides and between the two nuclei which push down into the chalazal region a wall immediately forms (Figs. 33, 39). The nucleus in the lower cell divides once without any wall forming between. Division of the upper nucleus continues, followed by the formation of cell walls, but after four to six cells have formed throughout the length of the sac their nuclei divide at right angles to the length of the sac thus forming two rows of cells (Figs. 40, 41, 46). This division proceeds from the chalazal region upward. Walls appear in various planes after this time so that the original walls are soon obscured.

In the meantime the sac elongates with the growth of the ovule,

the chalazal region increases in width and the whole region of the sac extending from the egg to the chalazal end fills up with endosperm cells. Endosperm nuclei are occasionally seen in the extreme micropylar end of the sac, but no cell walls appear (Fig. 41).

During endosperm formation the tapetum stains heavily and appears to be very active during the early development of the embryo.

This method of endosperm formation conforms to that of Schmid's second group in which he placed *Linaria* and *Antirrhinum* (20). *Striga lutea* also follows this method of endosperm formation (16).

Haustoria

A haustorium shaped like a bicuspid tooth is formed at the chalazal end of the embryo sac by the bifurcation of the lower endosperm cell which has at this stage become binucleate. One of the two nuclei moves down in each fork (Figs. 42, 43, 44). The prongs of the haustorium bending 35 degrees or more from the longitudinal direction of the sac penetrate the bed of nutritive tissue in the outermost tip of the ovule (Figs. 43, 44). These are in close touch with the food brought in through the chalazal region. By the time the embryo is well advanced the haustorial tissue breaks down (Fig. 48). Two endosperm nuclei at the upper end of the sac push up into the neck of the micropyle to form a two-legged micropylar haustorium (Fig. 35). These nuclei later become very large and stain conspicuously. Cross sections of this haustorium in its lower part show the two distinct parts of the basal prongs and frequently the two nuclei appear in this region (Fig. 37). The upper part of the haustorium forms a large bulbous structure which contains one large, or several smaller vacuoles and the two big nuclei (Fig. 36). The nuclei vary in position as they are found frequently either in the basal prongs or in the enlarged upper region. The haustorium consumes a considerable portion of the integumentary cells lying around the micropyle for the benefit of the developing embryo. Later when its work is completed, its structure collapses and only a porous mass remains (Fig. 47).

There is no tapetal layer in the upper region of the sac. However, at the time when the micropylar haustorium first develops, the cells of the first integumentary layer in the uppermost region of the sac and extending into the neck of the micropyle seem quite well defined and distended (Fig. 45). These probably function as tapetal cells for a short time. Such a condition is reported to be the case in *Scoparia dulcis* (2).

EMBRYO

The fertilized egg rests until the embryo sac is well filled with endosperm (Figs. 41, 42, 45). The first division of the egg is transverse, the lower cell giving rise to the embryo and the upper to a long suspensor (Fig. 50) consisting of about six cells (Fig. 51). It pushes the embryo about one-fourth of the way down into the endosperm. The first two divisions of the embryo proper are longitudinal and at right angles to each other (Figs. 51, 52). Further division proceeds rapidly and a many-celled globular embryo is soon formed (Fig. 53). The suspensor cells break down early and soon disappear.

The first indication of the cotyledons is seen as slight projections on the outer corners of the embryo which is by this time a little longer than broad (Fig. 47). The mature embryo has two cotyledons of approximately equal length which constitute slightly less than half the total length of the embryo (Fig. 54). It fills almost the entire endosperm region, being surrounded by only two or three layers of endosperm cells in the mature ovule (Figs. 61, 62).

The embryo may not develop even though the endosperm develops in the usual manner. A great many of the ovules do not have embryos. Slide after slide, each of which contained many sections through ovules whose sacs were filled with endosperm, was examined which showed only one or two embryos or quite frequently none in the whole number of ovules on a slide. Cook reports a similar observation for *Linaria vulgaris*, which also has many small seeds in a single ovary (7). He says that the majority of ovules in this species do not have embryos and that in some cases perhaps whole ovaries may be without them. Many mature seed of *Paulownia* appearing normal externally show upon examination with the low power of the microscope a mass of almost translucent endosperm tissue uniform in density throughout whereas those containing embryos show a dense, dark mass filling most of the embryo sac region.

CHANGES IN THE ENDOSPERM AND DEVELOPMENT OF THE SEED COAT

Soon after the haustoria begin to function the first two or three layers of endosperm cells in the haustorial regions begin to show by their denser protoplasm and greater capacity for stain that they are filling up with nutritive material. This storage of food material begins first in the chalazal region and is soon followed by the same behavior in the micropylar region (Figs. 47, 48). As growth continues, the accumulation of

food material extends to all the endosperm cells throughout the entire length of the ovule so that the growing embryo is surrounded by cells well supplied with food. The mature embryo is surrounded by a few collapsed endosperm cells which have been exhausted of food and crowded together, and by two or three layers of endosperm cells around the outside that remain gorged with food material to be used later for nourishing the seedling (Figs. 61, 62).

The seed coat consists of two layers which become differentiated from the cells of the integument (Figs. 61, 62). The layer of cells lying next to the embryo sac become lignified and are more or less quadrangular in cross section. This layer corresponds to what Bachmann has termed the quadratic, or inner layer (1). The walls of these cells become considerably thickened, and in fact, in the mature seed, seem to make up the greater part of the cell (Fig. 60). Despite the thickening of the walls this layer often appears somewhat sinuous due to slight infoldings into the endosperm. These slight curves do not noticeably affect the outer surface of the seed. The walls of the inner layer become slightly brownish upon ripening.

The outer layer of the seed coat consists of almost transparent cells. The outer cells covering the two longitudinal surfaces of the ovule follow the general shape of the inner cells, the tangential diameter being the greater. The end walls are slightly thickened so that the ends of the cells where they meet are fairly well supported. The ends of adjoining cells are similarly thickened throughout the length of the ovule, but the outer surface walls of the cells being unsupported in any way tend to sag and this accentuates the elevations at the ends of the cells so that there appear to be a few faint longitudinal striations on the outer surface. Along the sides of the ovule a few cells expand outward to varying lengths so that the outermost margin is not more than two cells in thickness thus forming light delicate appendages resembling somewhat the wings of a small insect. All the walls of the cells of the outer layer except the outer surface are strengthened by net thickenings. Viewed from the outer surface these thickenings in the cell walls appear as in Figure 58, or when looked at in cross section as in Figures 59 and 62.

THE SEEDLING

Paulownia seeds planted in soil germinate within a few days. The tiny seedling pushes through the soil with its two cotyledons often still enclosed in the old seed coat, but by the time that it is a centimeter or

so tall the cotyledons expand enough to push off the seed coat entirely. The seedling sends down one little root which has a scanty supply of root hairs just below the surface of the soil. It is exceptionally small and delicate, appearing much less sturdy than a radish seedling, and grows slowly. Some seedlings grown in soil in the laboratory were barely 1.5 cm. tall when three weeks old and the cotyledons, or first leaves, were not much larger than they were at the time when the seed coat was shed. This delicate seedling is in odd contrast to the superlatively vigorous tree which grows like Jack's bean-stalk. Stump shoots have grown 21 feet in one season.

SUMMARY

1. *Paulownia tomentosa* Steud. is an introduced plant belonging to the sub-family Antirrhinoideae of the Scrophulariaceae. It grows to tree size.

2. The microsporangia develop as usual. The wall of the sporangium has an epidermal layer, three to four wall layers, and a tapetal layer.

3. The microspore tetrad is developed in the usual way.

4. The microspores are formed in September.

5. The 2-nucleate male gametophytes were observed in October. These spend the winter in this stage.

6. The generative nucleus does not divide before the shedding of the pollen.

7. Artificially germinated pollen grains showed only the tube nucleus and the generative nucleus in the pollen tubes.

8. The pollen grain has three pores in the exine for the exit of the pollen tube.

9. The ovary is bilocular and the ovules are anatropous with one thick integument.

10. The megaspore mother cell is formed directly from a subepidermal cell in August. As usual it produces four megaspores, the lower one forming the embryo sac.

11. The 8-nucleate embryo sac develops as usual. It contains one large egg, two synergids, two polar nuclei, and three small antipodals which degenerate early.

12. The micropylar end of the sac becomes bulbous while the chalazal end becomes long and narrow. The polar nuclei fuse in the mid-region of the sac just within the bulbous region.

13. Pollen tubes enter the embryo sac, but do not penetrate the egg cell.

14. Double fertilization occurs.
15. The nucellus consists of a single layer of cells which surround the megaspore, and break down early.
16. A single layer of tapetal cells develops around the chalazal portion of the sac. It begins to be differentiated at the time the functional megaspore divides.
17. The first layer of cells of the integument in the micropylar region functions as a nutritive layer during the formation of the micropylar haustorium.
18. Endosperm is formed by cell division and begins before the fertilized egg divides.
19. There is a large mass of nutritive tissue in the chalazal region of the ovule and a small one around the base of the micropyle. A long, curved, two-pronged, binucleate haustorium forms at the chalazal end and penetrates the nutritive tissue there, while a large bulbous haustorium protrudes into the micropyle and is surrounded in its lower region by a small mass of nutritive tissue.
20. The proembryo has a suspensor of about six cells.
21. The mature embryo has two cotyledons and is surrounded by two to three layers of endosperm cells gorged with food materials.
22. A large percentage of ovules do not develop embryos, but the endosperm develops normally even in the absence of an embryo.
23. The seed coat consists of two layers of cells, an inner layer of thick-walled lignified cells, and an outer almost transparent layer which is expanded on each side to form wing-like outgrowths.
24. The seedling is exceptionally small and delicate.

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EXPLANATION OF PLATES

All drawings were made at table level with the aid of a Spencer camera lucida and reduced. The figures are so placed that the micropylar end of the embryo sac is turned toward the bottom of the page.

Abbreviations: *ant.*, antipodals; *c.h.*, chalazal haustorium; *cot.*, cotyledon; *e.*, egg; *emb.*, embryo; *emb. s.*, embryo sac; *emb. c.*, embryo cells; *end. c.*, endosperm cells; *ep.*, epidermis; *f. n.*, fusion nucleus; *fun.*, funiculus; *g. c.*, gelatinized cells; *l. c.*, lignified cells; *mic.*, micropyle; *m. h.*, micropylar haustorium; *n. t.*, net thickenings; *nuc.*, nucellus; *nut.t.*, nutritive tissue; *o.l.*, outer layer; *p.s.op.*, pollen sac opening; *p.t.*, pollen tube; *s.*, sperm; *sp.t.*, sporogenous tissue; *sp.m.c.*, spore mother cell; *sus. c.*, suspensor cell; *s.w.*, seed wing; *syñ.*, synergid; *t.*, tapetum; *t.f.n.*, triple fusion nucleus; *t.n.*, tube nucleus.

PLATE 4

Paulownia tomentosa Steud.

1. Section of anther showing differentiation of sporogenous tissue; $\times 266$.
2. Sporogenous tissue of four layers surrounded by tapetal cells; $\times 585$.
3. Spore mother cells surrounded by tapetal cells some of which are binucleate; $\times 266$.
4. Diad stage of spore mother cells which are enclosed by large, binucleate tapetal cells, four parietal layers, and epidermis; $\times 266$.
5. Tetrad stage of spore mother cells; $\times 266$.
6. Segment of anther wall of March 10th showing tapetal cells still present and binucleate spores; $\times 266$.
7. Segment of anther wall of April 11th showing complete disappearance of tapetum; $\times 60$.
8. Spore mother cell with spindle in late metaphase stage in preparation for the diad stage; $\times 585$.
9. Diad stage of spore mother cell; $\times 585$.
10. Spindles preceding the formation of a tetrad; $\times 585$.
11. Tetrad of spores within old mother cell wall; $\times 585$.
12. Mononucleate spore of September 4th; $\times 585$.
13. Binucleate spore of October 17th; $\times 585$.
14. External view of mature spore; $\times 585$.
15. Pollen tube sprouted in 5% sugar solution showing tube nucleus and generative nucleus (fixed by Carnoy's fluid and stained by Feulgen's method); $\times 585$.
16. Half of cross section of anther showing location of two of the four pollen sacs and point of dehiscence; $\times 30$.

17. Archegonial mother cell in young ovule. Origin of integument becoming visible; $\times 585$.
18. Archegonial mother cell in synapsis. Indications of nuclear activity in cells of integument of March 10th; $\times 585$.
19. Unusual case of three archegonial cells in one ovule in synapsis on March 10th; $\times 585$.
20. Two cells formed by first division of megaspore mother cell enclosed by a 1-layered nucellus; $\times 266$.
21. Four megaspores surrounded by nucellus; $\times 266$.
22. Lower functional megaspore with three upper megaspores in stage of degeneration; $\times 585$.
23. Two-nucleate embryo sac. Remnants of three upper megaspores present; $\times 585$.
24. Four-nucleate embryo sac; $\times 585$.
25. Chalazal end of embryo sac showing three antipodal cells, and lower polar nucleus. The degenerating nucellus and a well developed tapetum adjoin this region; $\times 585$.
26. Four nuclei in micropylar end of sac. Polar nucleus distinctly larger than others. $\times 573$.

PLATE 5

Paulownia tomentosa Steud.

27. Eight-nucleate embryo sac. Egg apparatus organized. $\times 285$.
28. Seven-nucleate sac showing fusion nucleus and degenerating antipodals; $\times 285$.
29. Fusion nucleus in region of the egg apparatus; $\times 585$.
30. Double fertilization. One sperm nucleus is just above the fusion nucleus and the other is near the egg nucleus; $\times 573$.
31. Two sperms lying over the egg and in the region of the egg nucleus. One synergid still intact. $\times 573$.
32. Triple fusion nucleus in position for movement away from the fertilized egg and remaining synergid; $\times 573$.
33. Pollen tube in neck of micropyle and sperm nucleus within the egg. Two endosperm nuclei in lower part of sac with indication of wall formation between them. $\times 285$.
34. Cross section of ovule showing epidermis, integument, tapetum, nucellus and embryo sac; $\times 266$.
35. Binucleate micropylar haustorium. Group of nutritive cells at base of micropyle. $\times 285$.
36. Cross section through bulbous portion of micropylar haustorium containing two nuclei and surrounded by nutritive cells; $\times 285$.
37. Cross section through lower part of micropylar haustorium showing two distinct parts, each containing a nucleus; $\times 285$.
38. Micropylar haustorium with very large vacuole and two big nuclei. Endosperm cells adjoining the haustorium. $\times 285$.
39. Two endosperm nuclei in chalazal region of sac. Remnants of antipodals and nucellus still present, but their nuclei almost degenerated; $\times 285$.
40. Four endosperm nuclei. One cross wall has formed and the lower cell gives evidence of bifurcation. $\times 285$.

41. Embryo sac containing four endosperm cells, a fertilized egg, and two free endosperm nuclei in micropylar end of sac; $\times 285$.
42. Similar to Fig. 40 except that the lower chalazal cell is partly bifurcated. The fertilized egg and one endosperm nucleus in the micropylar region. $\times 285$.
43. Two-pronged chalazal haustorium. Endosperm nuclei dividing at right angles to the length of the sac and walls forming between; $\times 285$.
44. Chalazal haustorium surrounded by mass of small nutritive cells in tip of ovule; $\times 285$.
45. Embryo sac containing two rows of endosperm cells and a fertilized egg. Section of micropylar haustorium in micropyle. First layer of integumentary cells conspicuous around upper portion of sac. $\times 160$.
46. Stage similar to Fig. 45. Fertilized egg conspicuous. $\times 285$.

PLATE 6

Paulownia tomentosa Steud.

47. Micropylar end of older ovule showing collapsed micropylar haustorium, mass of nutritive cells around neck of micropyle, gelatinized cells at entrance to micropyle, outer rows of endosperm cells containing surplus food material, layer of lignified cells indicated by dark line, and embryo with beginning cotyledons. $\times 30$.
48. Chalazal end of older ovule showing complete disappearance of haustorium, mass of nutritive cells in tip of ovule, and endosperm cells in chalazal part of sac containing surplus food material; $\times 266$.
49. Fertilized egg beginning to enlarge; $\times 573$.
50. First division of fertilized egg forming first cell of embryo and suspensor cell; $\times 573$.
51. Young embryo with 6-celled suspensor; $\times 585$.
52. Cross section of 4-celled embryo; $\times 585$.
53. Advanced embryo now globular in shape. Nuclei of suspensor cells disintegrating. $\times 585$.
54. Outline of older embryo showing cotyledons; $\times 60$.
55. Section through young ovule showing its shape at the time when the megaspores are present; $\times 585$.
56. Shape of ovule at time of endosperm and haustoria formation; $\times 69$.
57. Longitudinal section through young seed; $\times 30$.
58. Appearance of net thickening of wing of seed from surface view of portion of two cells; $\times 285$.
59. Cross section of outer margin of wing showing net thickening of cell walls; $\times 285$.
60. Inner surface view of group of cells from lignified layer of seed coat; $\times 160$.
61. Section from a mature seed showing an outer layer, an inner layer of lignified cells, several layers of endosperm cells, and a section of the embryo; $\times 285$.
62. Cross section of mature seed showing cells of wings with net thickenings, layer of lignified cells, endosperm cells, and cotyledons; $\times 41$.

PLATE 4

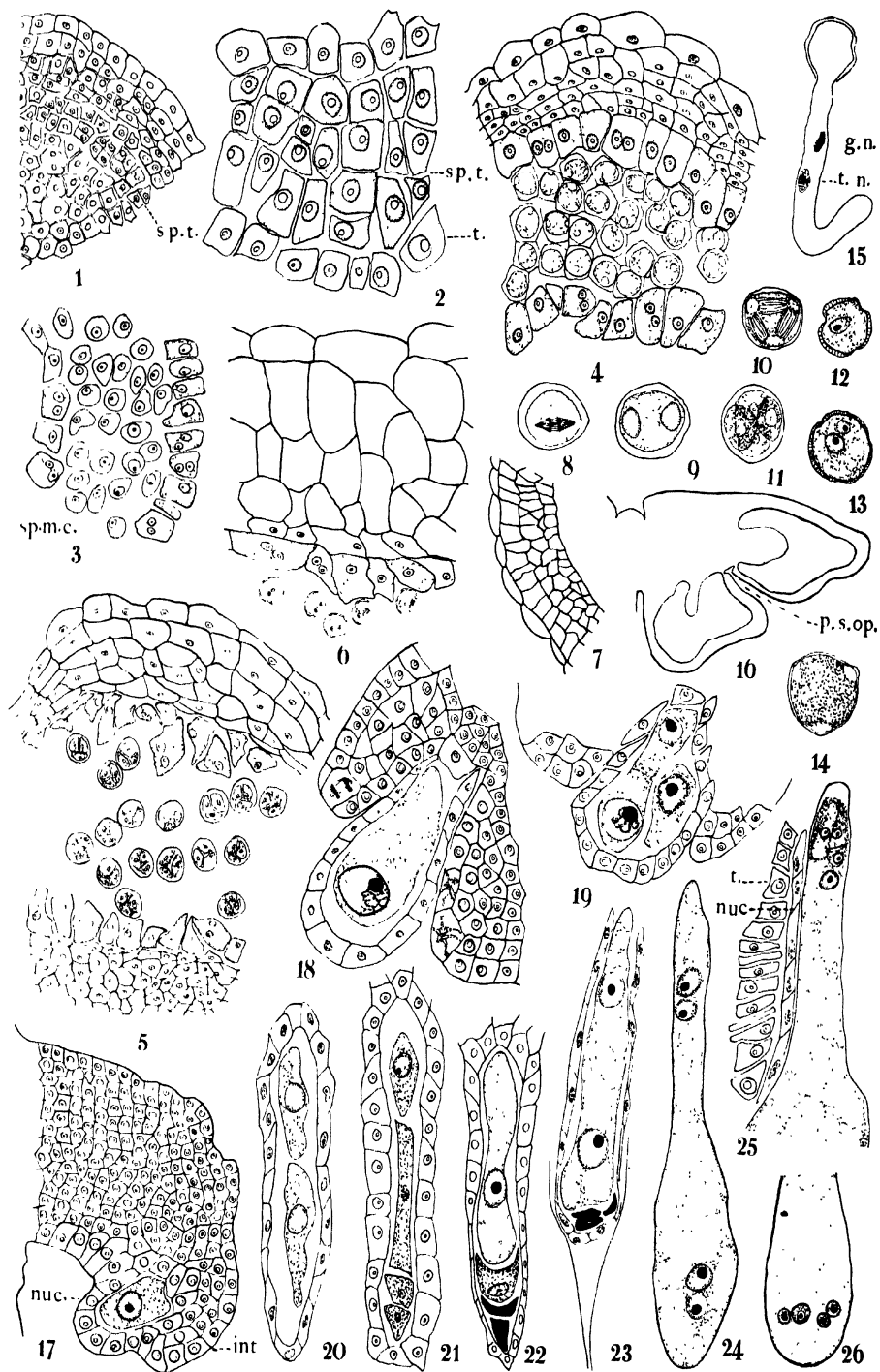


PLATE 5

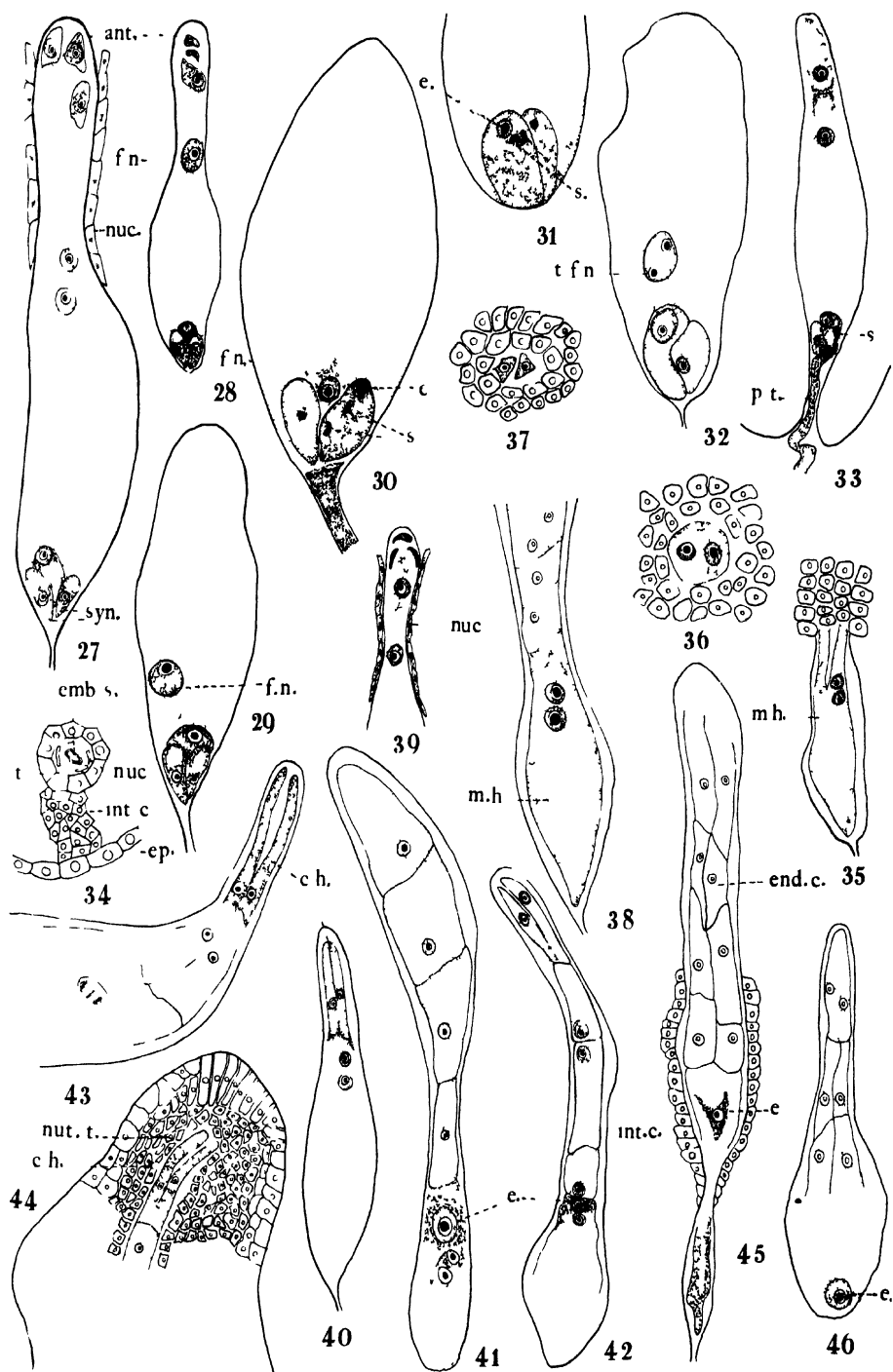
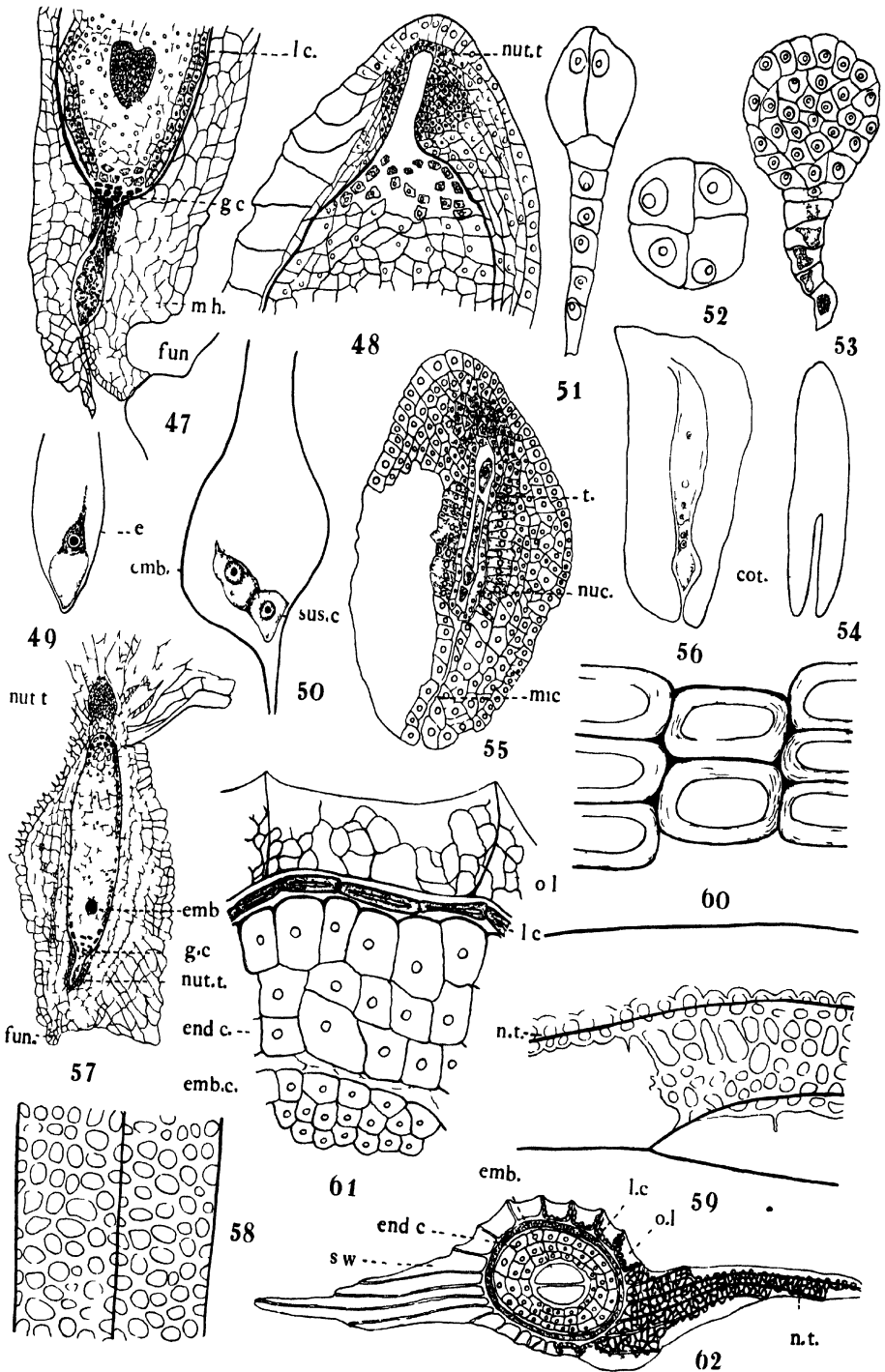


PLATE 6



A FIELD TRIP TO THE DEVIL'S MILLHOPPER¹

By LILLIAN E. ARNOLD

PLATES 7-10 AND 1 TEXT FIG

An interesting locality for a botanical study is found at the Devil's Millhopper,² a large, deep limestone sink located six miles northwest of Gainesville, Florida. Its fascination is such that it has become a mecca for visiting botanists at all times of the year. The sink can be detected from a distance by the dense growth of woody plants which attracts the eye amidst an area of broad, flat, cut-over and burnt-over pineland on which *Quercus Catesbaei*³ has become established. Closer inspection reveals that the vegetation here differs very greatly from that of the surrounding territory. Its interest lies in this thick growth of an extensive mesophytic and semi-hydrophytic flora at a station situated in an area of more or less semi-xerophytic type. Although sinks are not uncommon in this region of the state, it seldom happens that their vegetation differs so strikingly from that of the surrounding territory, as it usually takes its type from the area in which the sink occurs. Sinks occurring in hammocks are usually occupied by hammock vegetation, while those in scrubby regions maintain a scrubby type of vegetation.

PHYSIOGRAPHY

The age of a sink hole can be judged by its shape, as new ones have almost perpendicular sides. Gradually plants move into the sides, but until the woody plants become established, the sides will continue to

¹ During the period extending from the fall of 1929 through the spring of 1933, a floristic study was made of the ferns and higher plants which occur conspicuously at this station and the complete study, entitled "A Study of the More Conspicuous Flora of the Devil's Millhopper," was presented in partial fulfillment of the requirements for the degree of Master of Science at the University of Florida in January, 1934.

² In June 1935, forty acres of land including the Millhopper were purchased by the Junior Chamber of Commerce of Gainesville with a view of making it into a public park.

³ Nomenclature of Spermatophyta follows: Small, John K. Manual of the Southeastern Flora. 1554 pp. Illus. Author, 1933.

shelve, because the early plant associations do not bind the soil sufficiently to check further talusing. However, over the course of many years erosion is bound partly to fill the cavity, so that very old sinks can be recognized by their gently sloping sides and shallowness, as well as by the fact that the outlets for drainage have become clogged.

A survey of the Devil's Millhopper has shown that it is approximately 114 feet deep, 500 feet in diameter at the top and 60 feet at the bottom. Its banks incline at angles between 30° and 35° with the horizontal. About half way down the incline, there is a ledge 25 to 30 feet wide, which extends practically all the way around the sink and forms a good point of vantage from which to view the base. On the basis of these facts, the Devil's Millhopper is an old sink; probably as much as one thousand years.

The very prominent ledge which encircles the sink at about half its depth indicates that there probably have been two distinct cave-ins. This theory seems to be substantiated by the presence of several specimens of very large trees that stand in the area between the ledge and the perimeter of the sink, for these trees, which will be discussed later, have proved by measurement to be the largest of their species growing anywhere in this sink. The first cave-in may have occurred to this depth, and the action of the water continued to deepen it to its present level. Even now the cavity does not extend as deep as the surface of the water-table, because the water does not stand in it. An interesting subject for conjecture is whether further erosion will cause another cave-in to the depth of the water-table, or whether debris will accumulate and fill it. This will depend upon the composition of the soil underlying its present bottom.

A geological study was made of this station by Cooke and Mossom⁴ and their data are as follows: "The Devil's Millhopper, a sink 6 miles northwest of Gainesville, cuts through at least 115 feet of Hawthorn beds to the Ocala limestone, which doubtless lies not far below water level. The following section at the Devil's Millhopper was measured by Cooke in 1913:

Hawthorn formation:

	Feet
10—Covered; debris of calcareous sandstone.....	25
9—Gray or cream-colored calcareous sandstone or sandy limestone containing round phosphatic grains, poorly preserved mollusks, and echinoids; lower part more sandy and paler than upper; this bed has slumped several feet.....	15

⁴ Cooke, C. Wythe, and Stuart Mossom. *Geology of Florida*. 20th Ann. Rept. Fla. State Geol. Survey, 129. 1929.

	Feet
8—Concealed; partly encrusted with travertine.....	27
7—Green sandy clay, upper part encrusted with travertine 1 inch to more than 4 inches thick and enclosing land shells.....	9
6—Hard silicified green clay or fuller's earth.....	5
5—Greenish-gray sand and fuller's earth.....	15
4—Covered.....	
3—Hard cream-colored or yellow phosphatic fossiliferous limestone at base passing upward into sandy phosphatic limestone; probably a fallen block of bed 9.....	11
2—Soft calcareous sand with brown phosphatic pebbles and moulds of fossils; seen below waterfall on west side.....	2
1—White calcareous sandstone with phosphatic nodules; to water level.....	5"

From this study it is noticeable that most of the soil in this station is of calcareous origin.

METEOROLOGICAL CONDITIONS

The banks of this sink from the outer rim to the ledge are so heavily wooded for the entire circumference, that only a filtered light reaches the forest floor of this area. From the ledge to the bottom of the incline, the stand of trees is thinner, which permits direct sunlight to penetrate more readily, except on the south side with the northern exposure. The east, north, and west sides receive a little direct sunlight at some time of the day, while the mud bottom revels in sunlight from the time the sun shines over the tree tops on the east until it sets behind the tree tops.

The largest waterfall is located on the west side, where a surface drainage stream has worn a small ravine down to the ledge, from whence it splashes over the rocks in a succession of falls to the bottom. Here it wanders in a northerly direction until the water disappears under a large rock on the north side of the bottom. Two seepage streams appear as springs at or near the ledge on the south and southwest sides, from whence the waters fall to the bottom in two series. During the four-year period of study, these three streams were never observed to have dried up entirely. During very rainy periods seepage water oozes into the sink on all sides, but does not accumulate into well-defined streams.

During the years 1931-32 and 1932-33, there was an intermittent drought on the eastern seaboard and particularly in Florida. In these two particularly dry years the water of the spring freshets soon vanished from sight through the underground outlet indicating that the

water-table had fallen. During this period of extreme drought the principal points of drainage into the underground stream were apparent. While there seems to be a certain amount of seepage draining into the cavity from half a dozen sources, still the water from these sources is never sufficient to cause more than a small stream at the bottom. In times of abnormal rainfall the water in the sink may reach a depth of approximately 15 feet, as indicated by the growth of perennial plants on higher levels.

When the bottom is completely or partly filled with water, there are no plants on the floor, yet it is exceedingly interesting to note the number and variety of annuals and the few perennials that spring up as soon as this pool is reduced to the small drainage stream. During the years, 1931-32, 1932-33, it was so dry that the bottom of this sink became solid enough to walk upon and the invasion of the pioneer flora, which is fully waist high in some places, has bound the soil even more firmly. Thus, this bottom growth here forms a distinct contrast to the plant associations occurring on the banks at higher levels. These banks are thickly wooded with a variety of very fine specimens of trees, while the floor of this forest is heavily covered with ferns, especially on the south side which lacks direct sunlight.

In February 1933 a fire in the pine flats at the north of this sink crept into it as far as the ledge on the north side. *Dryopteris* and *Rhapido-phyllum* specimens were badly injured, but were not killed. Several oaks were scarred so badly that they show signs of dying. However, taken as a whole, the damage was slight.

FLORISTICS

Floristic studies naturally resolve themselves into investigation of the four regions or zones delimited by the topography of the station. These zones (Fig. 1) are based solely on the topography and do not seem to bear any relation to the geological strata. Although there are no clear-cut marks of definition between these zones, still certain species occur in one zone that do not occur in others, as shown in Table 1. However, only the most conspicuous species are pointed out in the following discussion.

In order to secure an idea of the relationship of the flora of this station to that of the territory immediately surrounding it, the outer zone was arbitrarily defined to extend to a distance of 20 to 25 feet from the perimeter of the sink and has been designated as Zone 1. The area from the abrupt rim of the sink to the ledge has been designated as

Zone 2. The banks extending from the ledge to the bottom of the incline have been designated as Zone 3, while the area of the recently exposed mud bottom, which has furnished a study of pioneer associations, has been designated as Zone 4. These four zones, based solely on greatly contrasting topographical conditions occurring in a distinctly limited area, afford the best means of making the ecological study of the flora within this station, as well as with that of the surrounding terrain.

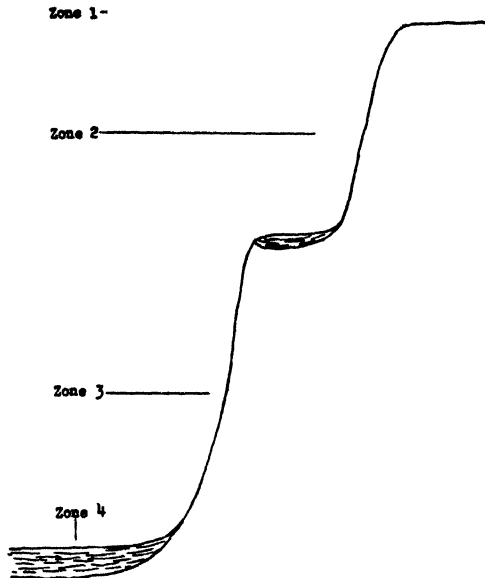


FIG. 1. DIAGRAMMATIC SKETCH SHOWING THE CONTOURS OF A SIDE OF THE DEVIL'S MILLHOPPER AND THE LOCATION OF ZONES

Zone 1

The car trail leading from the paved highway ends suddenly on the southern brink of the sinkhole under some large specimens of *Hicoria glabra* and a damaged individual of *Padus virginiana*, the only one at this station, that still stands toweringly with a few branches at the top. Measurements show that it has a girth of 6 feet 9 inches at a point 3 feet from the ground. Alighting from the car at this point, a walk around the periphery reveals much shrubby growth on the margin intermingled with many good-sized examples of *Nyssa sylvatica*, *Carpinus caroliniana*, *Quercus nigra*, and *Ostrya virginiana*, while much of the floor is carpeted with the mottled-leaved species of violet, *Viola Walteri*. The northeast and eastern sectors display interesting

TABLE 1

List of the flora of the Devil's Millhopper, showing distribution of species by zones
(S indicates sparing; M indicates moderate; A indicates abundant)

FAMILY AND PLANTS	ZONE			
	4	3	2	1
Polypodiaceae:				
<i>Polypodium Plumula</i> H. B. K...		S		
<i>Polypodium polypodioides</i> (L.) A. S. Hitch.		M		
<i>Pycnodoria Cretica</i> (L.) Small		A	A	
<i>Adiantum tenerum</i> Sw.		S		
<i>Asplenium resiliens</i> Kunze....			S	
<i>Asplenium heterochroum</i> Kunze..			S	
<i>Dryopteris floridana</i> (Hook.) Kuntze		A	A	
<i>Dryopteris normalis</i> C. Chr..		A	A	
Pinaceae:				
<i>Pinus taeda</i> L. .			S	S
<i>Pinus glabra</i> Walt. ..			S	S
Poaceae:				
<i>Paspalum longepedunculatum</i> LeC	A			
<i>Oplismenus setarius</i> (Lam.) Roem & Schult. . .		A	A	
<i>Panicum Joorii</i> Vasey ...		A	A	
<i>Trisetum pennsylvanicum</i> (L.) B S P.	A			
<i>Eleusine indica</i> (L.) Gaertn. ...	A			
<i>Uniola laxa</i> (L.) B. S. P. . .			S	
Cyperaceae:				
<i>Cyperus virens</i> Michx. . .	A			
<i>Kyllinga pumila</i> Michx. .	M			
<i>Carex stipata</i> Muhl. .	A			
<i>Carex dasycarpa</i> Muhl. ..				S
<i>Carex lupulina</i> Muhl. . .	A			
Arecaceae:				
<i>Sabal minor</i> (Jacq.) Pers. . . .			S	S
<i>Rhapidophyllum Hystrix</i> (Fraser) H Wendl.			S	S
Araceae:				
<i>Arisaema acuminatum</i> Small.		A	A	
<i>Muricauda Dracontium</i> (L.) Small		M	M	
Commelinaceae:				
<i>Commelina erecta</i> L.....			S	S
<i>Commelina angustifolia</i> Michx...				S
Bromeliaceae:				
<i>Dendropogon usneoides</i> (L.) Raf.				A
<i>Tillandsia tenuifolia</i> L.			S	

TABLE 1—Continued

FAMILY AND PLANTS	ZONE			
	4	3	2	1
Smilacaceae:				
<i>Smilax glauca</i> Walt.....		M	M	M
<i>Smilax auriculata</i> Walt.....		A	A	A
<i>Smilax laurifolia</i> L.....		A	A	A
<i>Smilax pumila</i> Walt.....		M	M	M
Tamaceae:				
<i>Dioscorea floridana</i> Bartlett.....			S	
Orchidaceae:				
<i>Ponthieva racemosa</i> (Walt.) C. Mohr.....			S	
<i>Malaxis spicata</i> Sw.....			S	
<i>Tipularia unifolia</i> (Muh.) B. S. P.....				?
<i>Amphiglottis conopsea</i> (Ait.) Small.....			S	
Saururaceae:				
<i>Saururus cernuus</i> L.....	S			
Juglandaceae:				
<i>Hicoria glabra</i> (Mill.) Britton.....				A
Salicaceae:				
<i>Salix longipes</i> Anders.....	S			
Corylaceae:				
<i>Carpinus caroliniana</i> Walt.....		A	A	A
<i>Ostrya virginiana</i> (Mill.) Willd.....		A	A	A
Fagaceae:				
<i>Quercus Prinus</i> L.....			S	M
<i>Quercus virginiana</i> Mill.....			S	M
<i>Quercus nigra</i> L.....				M
<i>Quercus laurifolia</i> Michx.....				S
Urticaceae:				
<i>Adicea pumila</i> (L.) Raf.....	A	M		
<i>Boehmeria cylindrica</i> (L.) Willd.....		A		
Artocarpaceae:				
<i>Morus rubra</i> L.....			S	
Ulmaceae:				
<i>Ulmus alba</i> Michx.....		S		
<i>Celtis georgiana</i> Small.....			S	
Polygonaceae:				
<i>Persicaria hydropiperoides</i> (Michx.)				
Small.....	A			
<i>Persicaria setacea</i> (Baldw.) Small.....	A			
Amaranthaceae:				
<i>Iresine Celosia</i> L.....	S			
Phytolaccaceae:				
<i>Phytolacca rigida</i> Small.....		S		M
Ranunculaceae:				
<i>Viorna reticulata</i> (Walt.) Small.....				S

TABLE 1—Continued

FAMILY AND PLANTS	ZONE			
	4	3	2	1
Annonaceae:				
<i>Asimina parviflora</i> (Michx.) Dunal....			S	
Magnoliaceae:				
<i>Magnolia grandiflora</i> L.....		A	A	A
Brassicaceae:				
<i>Radicula Walteri</i> (Ell.) Small.....	A			
Iteaceae:				
<i>Itea virginica</i> L.....		M		
Altingiaceae:				
<i>Liquidambar Styraciflua</i> L.....		A	A	A
Rosaceae:				
<i>Rubus floridus</i> Tratt.....		A		
<i>Rubus trivialis</i> Michx.....				A
<i>Agrimonia microcarpa</i> Wallr.....			S	
Amygdalaceae:				
<i>Prunus angustifolia</i> Marsh.....				M
<i>Padus virginiana</i> (L.) Mill.....				S
Cassiaceae:				
<i>Chamaecrista brachiata</i> Pollard.....				M
Fabaceae:				
<i>Baptisia leucantha</i> T. & G.....				S
<i>Erythrina herbacea</i> L.....				S
<i>Phaseolus polystachyus</i> (L.) B. S. P....		A	A	
<i>Meibomia grandiflora</i> (Walt.) Kuntze..		S		
<i>Lespedeza hirta</i> (L.) Ell.....				S
Oxalidaceae:				
<i>Xanthoxalis stricta</i> (L.) Small.....	M			
<i>Xanthoxalis corniculata</i> (L.) Small....	M			
Rutaceae:				
<i>Zanthoxylum Clava-Herculis</i> L.....			S	
Meliaceae:				
<i>Melia Azedarach</i> L.....			S	
Euphorbiaceae:				
<i>Bivonea stimulosa</i> (Michx.) Raf.....				S
Spondiaceae:				
<i>Toxicodendron radicans</i> (L.) Kuntze. ...		A	A	M
<i>Toxicodendron Toxicodendron</i> (L.) Britton.....		A	A	
Aquifoliaceae:				
<i>Ilex ambigua</i> (Michx.) Chapm.....			S	S
<i>Ilex opaca</i> Ait.....			M	
Celastraceae:				
<i>Euonymus americanus</i> L.....		A	A	

TABLE 1—Continued

FAMILY AND PLANTS	ZONE			
	4	3	2	1
Frangulaceae:				
<i>Ceanothus intermedius</i> Pursh.....	S			
Vitaceae:				
<i>Muscadinia rotundifolia</i> (Michx.)				
Small.....		A	A	
<i>Ampelopsis arborea</i> (L.) Rusby.....		A	A	
<i>Parthenocissus quinquefolia</i> (L.) Planch.		A	A	A
Tiliaceae:				
<i>Tilia heterophylla</i> Vent.....		M	M	
Malvaceae:				
<i>Sida rhombifolia</i> L.....	M			
<i>Sida carpinifolia</i> L. f.....				M
Cistaceae:				
<i>Crocanthemum corymbosum</i> (Michx.)				
Britton.....	S			
Violaceae:				
<i>Viola sororia</i> Willd.....	M	A		
<i>Viola septemloba</i> LeConte.....				S
<i>Viola Walteri</i> House.....			A	A
Passifloraceae:				
<i>Passiflora lutea</i> L.....			S	
Lauraceae:				
<i>Tamala Borbonia</i> (L.) Raf.....			S	
Nyssaceae:				
<i>Nyssa sylvatica</i> Marsh.....			S	
<i>Svida microcarpa</i> (Nash) Small.....			M	
<i>Cynoxylon floridum</i> (L.) Raf.....			A	
Ammiaceae:				
<i>Eryngium Baldwinii</i> Spreng.....	S			
<i>Sanicula Smallii</i> Bicknell.....			S	
<i>Sanicula canadensis</i> L.....		S		
<i>Sanicula floridana</i> Bicknell.....			M	
<i>Hydrocotyle umbellata</i> L.....	A			
<i>Hydrocotyle verticillata</i> Thun.....		M		
<i>Ptilimnium capillaceum</i> (Michx.) Raf..	A			
Vacciniaceae:				
<i>Polycodium neglectum</i> Small.....				S
Primulaceae:				
<i>Samolus floribundus</i> H. B. K.....	A			
Sapotaceae:				
<i>Bumelia lanuginosa</i> (Michx.) Pers.....			S	
Oleaceae:				
<i>Frazinus pauciflora</i> Nutt.....	S			

TABLE 1—Continued

FAMILY AND PLANTS	ZONE			
	4	3	2	1
Oleaceae (Continued):				
<i>Frazinus americana</i> L.....		M		
<i>Chionanthus virginica</i> L.....			S	
<i>Amarolea floridana</i> (Chapm.).....			M	
Spigeliaceae:				
<i>Gelsemium sempervirens</i> (L.) Ait. f.....				M
Asclepiadaceae:				
<i>Amphistelma scoparia</i> (Nutt.) Small...		M	M	
Dichondraceae:				
<i>Dichondra carolinensis</i> Michx.....	S			
Solanaceae:				
<i>Physalis pubescens</i> L.....	S			
<i>Physalis pruinosa</i> L. (?).....	S			
<i>Physalis barbadensis</i> Jacq.....	A			
<i>Physalis angulata</i> L.....	A			
<i>Solanum gracile</i> Link.....	S	A		
Boraginaceae:				
<i>Lithospermum tuberosum</i> Rugel.....		S		
Verbenaceae:				
<i>Callicarpa americana</i> L.....			S	M
Lamiaceae:				
<i>Trichostema dichotomum</i> L.....				S
<i>Salvia lyrata</i> L.....			S	
<i>Monarda punctata</i> L.....				S
<i>Hyptis pectinata</i> (L.) Poir.....				S
Acanthaceae:				
<i>Ruellia hybrida</i> Pursh.....				M
Bignoniaceae:				
<i>Anisostichus crucigera</i> (L.) Bureau....				S
Rubiaceae:				
<i>Houstonia procumbens</i> (Walt.) Standley.				S
<i>Cephalanthus occidentalis</i> L.....	S			
<i>Mitchella repens</i> L.....		A	A	A
<i>Galium pilosum</i> Ait.....	S	M	M	
Caprifoliaceae:				
<i>Sambucus Simpsonii</i> Rehder.....		M		
<i>Viburnum rufidulum</i> Raf.....				M
<i>Viburnum obovatum</i> Walt.....				M
<i>Phenianthus sempervirens</i> (L.) Raf.....				S
Valerianaceae:				
<i>Valeriana scandens</i> L.....	M	A	A	
Cucurbitaceae:				
<i>Melothria pendula</i> L.....		M	M	
Lobeliaceae:				
<i>Lobelia puberula</i> Michx.....				S

TABLE 1—*Concluded*

FAMILY AND PLANTS	ZONE			
	4	3	2	1
Ambrosiaceae:				
<i>Ambrosia elatior</i> L.....				S
Carduaceae:				
<i>Vernonia ovalifolia</i> T. & G.....				M
<i>Elephantopus tomentosus</i> L.....				M
<i>Eupatorium capillifolium</i> (Lam.) Small.				S
<i>Eupatorium compositifolium</i> Walt.....				S
<i>Eupatorium aromaticum</i> L.....			S	S
<i>Mikania scandens</i> (L.) Willd.....		A		
<i>Laciniaria gracilis</i> (Pursh.) Kuntze....				S
<i>Solidago Chapmanii</i> T. & G.....				S
<i>Sericocarpus bifolius</i> (Walt.) Porter..				S
<i>Leptilon canadense</i> (L.) Britton.....				S
<i>Pluchea petiolata</i> Cass.....	S			
<i>Gnaphalium spathulatum</i> Lam.....	S			
<i>Gnaphalium purpureum</i> L.....	S			
<i>Bidens pilosa</i> L.....				A
<i>Bidens bipinnata</i> L.....		S		
<i>Erechtites hieracifolia</i> (L.) Raf.....	S			
Cichoriaceae:				
<i>Hieracium Gronovii</i> L.....		S	S	S
<i>Mulgedium floridanum</i> (L.) DC.....		S	S	M

groups of *Viburnum* and *Prunus*. The wide variation of leaf sizes and shapes of *V. rufidulum* present material for speculation. Several specimens have such large leaves that they simulate the appearance of cultivated pear trees, while the remainder have leaves about half as large. Another observation that can be made after the conclusion of the trip is that the composite population lies in this zone, a fact that leads one to decide that it is due to an invasion from the surrounding pineland.

Zone 2

The most frequented path of descent starts at a point where one leaps over a large fallen oak and proceeds to tumble down the steep incline of Zone 2 to the encircling ledge, struggling to maintain a foothold by planting the feet in exposed roots and by clutching tree trunks. It proves to be a sporting venture immediately after a shower, since there is enough clay in the soil to make the path slippery. Other paths (Fig. 2) meander downward at several points on the upper slope.

The forest floor of this area is heavily carpeted with *Dryopteris normalis*⁵ and *D. floridana*. It is difficult to draw conclusions about their choice for light and shade. Their dense growth in Zone 2 occurs on the south side where there is very little opportunity for receiving any direct sunlight. The predominant shrub of this zone is *Euonymus americanus*, which droops its long spreading stems over the water courses in company with the fern, *Pycnodoria cretica*. Of the trees, the two ironwoods, *Carpinus caroliniana* and *Ostrya virginiana*, together with the flowering dogwood, *Cynoxylon floridum*, appear to be predominant. Another outstanding plant is the bullace or muscadine grape, *Muscadinia rotundifolia*. Some of them are old, yet still vigorous individuals with stems as much as six inches in diameter at the base. One stem of this species, arising at the base of the cliff on the north side, clambers up a tree on the upper level of the sink. Judging by the tracks on the cliff, this has been used frequently as a living rope ladder for the elevation of the more agile visitors to the Millhopper. There are several fine specimens of sweet gum, *Liquidambar styraciflua* in this zone, one of which stands on the ledge on the south side (Fig. 3). It measures 10 feet 3 inches in circumference at a point 3 feet from the ground. The presence of this specimen growing so far below the elevation of the surrounding territory is another reason for geologists to believe that this limesink is quite old. Another specimen of remarkable proportions growing in this zone is an example of *Pinus glabra*, having a circumference of 7 feet 8 inches, which may be noted on the southeast bank near the ledge. One specimen of *Quercus virginiana*, whose limbs are covered with *Polypodium polypodioides*, grows in the rich soil of the ledge on the north side of this zone within the *Rhapidophyllum Hystrix* association that was burned in the spring of 1933. Its trunk measured 5 feet 9 inches in circumference at a height of 3 feet from the ground. One excellent specimen of *Q. Prinus* growing in a similar situation on the southeast side of this zone has a trunk that measures 8 feet 7 inches in circumference at the same point of measurement. All of these observations serve to substantiate the theory that the first cave-in may have extended to the ledge and thus have formed the original sink.

Zone 3

After reconnoitering the ledge, two paths of descent to the bottom are observed. The first is located close in line with the path from the

⁵ Nomenclature of Pteridophyta follows: Small, John K. Ferns of Florida. 237 pp. 107 illus. Science Press. 1931.

top and tumbles down alongside of a small gully washed from a spring (Fig. 4). In very wet weather it is impassable on account of steepness and the character of the soil, which is composed largely of clay and fuller's earth at this point. The easier means of descent is a path reached after walking a hundred years or so to the west around the ledge. The end of the path lies at the foot of a tree stump located on a bank which is above water level when the pool is filled.

In Zone 3 there is a special point of interest in a large *Dryopteris* colony (Fig. 5) growing on the bank having a southern exposure, where it has the advantage of direct sunlight and little competition, since there is an absence of perennials. Apparently, there has been a slide here in some not far distant time. This *Dryopteris*-covered slide is one of the paths of descent to the bottom, which is further accomplished by grasping the tough and tenacious fronds and sliding along. On arrival at the bottom of the cruise, one's feet land in oozing ground bearing large elderberry bushes and blackberry vines, while the floor is thickly populated with *Viola sororia*, which thrives prodigiously in this environment. As early as the last of January the inner periphery of this zone on the north side is a solid mass of pale violet blue. On the lower banks in moist locations, *Solanum gracile* has abandoned its upright habit of growth and droops long branches several feet toward the declivity.

Zone 4

Ideal conditions obtain in this zone for the establishment of a pioneer flora and many such plants have appeared during these years of drought. When the bottom became exposed, a small colony of *Salix longipes* sprang up in the mud. A few plants of *Saururus cernuus* appeared suddenly and also a small whip of *Cephalanthus occidentalis*, hydrophytic plants that are not found among the semi-xerophytic associations in the surrounding territory. On the western side at the foot of the largest waterfall, there is a heavy growth of the violet, *V. sororia*, previously discussed, while not far distant from this colony there is a thick mat of *Dichondra carolinensis*. Among the straggling specimens of *Iresine Celosia*, *Persicaria hydropiperoides*, *P. setacea*, and *Pluchea petiolata* are wound the stems of the twining plant *Valeriana scandens*.

Predominant in this association are the sedges and grasses (Fig. 6) with two species of *Carex* in preponderance. Great rank stems topped by the puffy bracts that characterize *C. lupulina* are intermingled with plants of *C. stipata*. Along the edges of the drainage streams are

growing *Adicea pumila* and straggling specimens of several species of *Physalis*, while in the streams themselves are *Radicula Walteri*, bishops weed, *Ptilimnium capillaceum*, and *Samolus floribundus*.

SUMMARY

A study of the more conspicuous flora of the Devil's Millhopper, a lime sink located six miles northwest of Gainesville, Florida, reveals that there are 160 species of Pteridophyta and Spermatophyta represented by 70 families of 38 orders.

Ecologically there are three plant associations in the form of circular zones here. The first is a semi-mesophytic association of plants that prefer the dry sand of the periphery of the sink among which certain viburnums and composites are typical; the second is the hardwood hammock association growing on the calcareous soils of the steep banks in which *Dryopteris* and *Quercus* predominate; and the third is the grass-sedge association which has developed on the alluvial soil of the recently exposed bottom.

Not only the topographical features, but also moisture and light are governing factors in the delimitation of these associations. The less mesophytic association on the periphery of the sink receives plenty of light, but suffers from excessive drainage due to the proximity of the steep walls of the depression. The hardwood hammock association receives the benefit of plenty of moisture from the seepage streams running in from all sides, yet the competition for light is very keen. The grass-sedge association responds luxuriantly to a generous supply of sunlight and moisture on the rich alluvial substratum.

Inasmuch as the type of vegetation in any given area is influenced primarily by the topography, the changing conditions at this station continually interfere with normal succession and practically preclude the attainment of a climax flora.

The writer wishes to acknowledge the assistance of Mr. David G. A. Kelbert in making the photographs.

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FIG. 2 (ABOVE) TYPICAL HARDWOOD VEGETATION IN THE DEVIL'S MILLHOPPER ALONG ONE PATH OF DESCENT IN ZONE 2

FIG. 3 (BELOW) VIEW OF LEDGE SHOWING FINE SPECIMEN OF LIQUIDAMBAR STYRACIFLUA WHICH STANDS ON THE SOUTH SIDE OF ZONE 2

PLATE 8



FIG. 4 DEVIL'S MILLHOPPER VIEW SHOWING TYPE OF VEGETATION GROWING ON THE SOUTH SIDE OF ZONE 3

The course of a seepage stream terminates in an underground outlet at the foot of the rock in the foreground.

PLATE 9



FIG. 5. DEAN S. MILLHOFFER. VIEW OF A COLONY OF *DRYOPTERIS NORMALIS* GROWING IN DIRECT SUNLIGHT ON THE NORTH SIDE OF ZONE 3.

PLATE 10

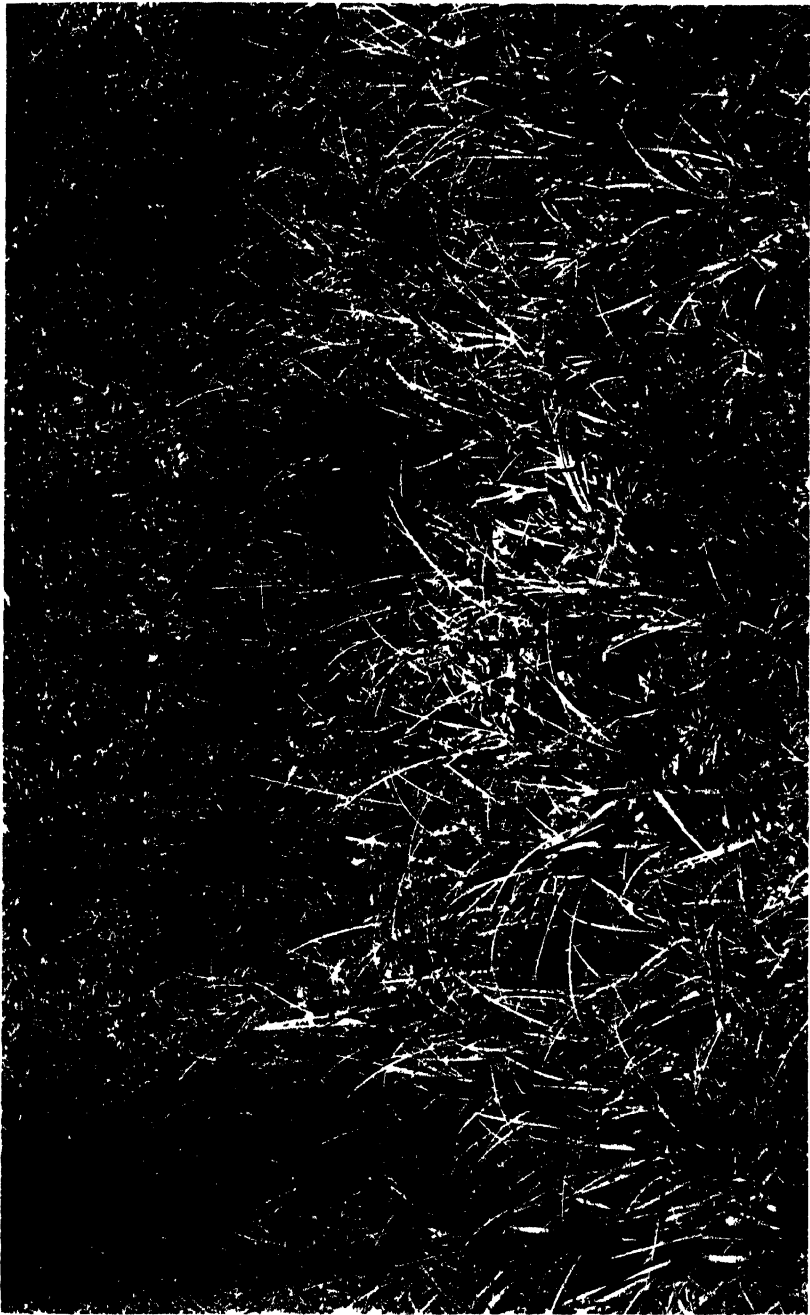


FIG. 6. VIEW OF THE GRASS-EDGE POPULATION OF ZONE 4 MAY BE SEEN IN THE FOREGROUND

EPIDENDRUM CONOPSEUM IN NORTH CAROLINA

By DONOVAN S. CORRELL

PLATE 11

Epidendrum conopscum R. Br. was reported for the first time from North Carolina by Thomas F. Wood and Gerald McCarthy in their *Wilmington Flora*, published in the *Journal of the Elisha Mitchell Scientific Society*, vol. 3, 1885-86. Credit is here given to a Major Young as having found it in Pender County in 1881. As far as the writer has been able to determine, there is no other record of this orchid having been found in the state until 1933 when Small included North Carolina in its (*Amphiglottis conopsea* (Ait.) Small) range in his *Manual of the Southeastern Flora*.

It is interesting to know that for a period of 46 years this orchid has evidently escaped collectors in North Carolina. In a conversation with Professor Oakes Ames last winter he expressed the opinion that the reason for this was perhaps due to the "Big Freeze" of 1888-89 which may have destroyed these plants this far north and thus temporarily moved the limits of its northern range farther south. It is also possible that the position of the orchid high up in the trees may have made it easily overlooked.

While collecting in the eastern part of the state last summer, much time was spent in an unsuccessful attempt to find new stations for *Epidendrum conopscum*. Not being discouraged, however, further search for this plant was made on the week-end of October 5 and 6, when Dr. H. L. Blomquist and the writer made a collecting trip through the Coastal Plain region of the state. On the return trip, a brief stop was made at Lake Waccamaw, Columbus County, to collect the Florida shield fern (*Dryopteris floridana* (Hook.) Kuntze) which reaches its northern limit there, and to look for any orchids which might be in flower at this time of the year.

After having found a few beautiful specimens of *Spiranthes odorata* (Nutt.) Lindl. in full bloom, growing in the flooded, mucky soil of a swamp on the northwestern side of the lake, we walked a short distance along a narrow, wooded sandbar, lying between the lake and the flooded swamp. Suddenly Dr. Blomquist called our attention to a large limb

of a sweet gum tree, growing out about 15 feet from the base of the trunk of the tree and arching out over the sandbar to about 8 feet from the ground. With that astonishment and delight experienced only by searching botanists, we saw growing on a considerable part of the limb a mantle of *Epidendrum conopseum* together with the epiphytic fern *Polypodium polypodioides* (L.) Watt. Needless to say, there was a clambering up the branch in a flash. At last, after many weeks of vain search and disappointments, here was success!

In spite of the inclement weather, the late hour of the day, the difficult position of the plant, and a single film left, we succeeded in obtaining a fairly respectable photograph of the orchid, which is reproduced herewith.

After returning home, we communicated with Dr. J. K. Small who kindly informed us that his report was based on a specimen collected by Mr. H. A. Rankin of Fayetteville, near Hallsboro, Columbus County. In response to a letter to Mr. Rankin, we have received a specimen from him with the following note: "We were cutting timber in White Marsh, a large swamp between Hallsboro and Whiteville (Columbus County), along the railroad crossing. On practically all of these large tupelo trees there were large colonies, growing just like *Polypodium* and frequently with this. All was high in the trees—from 40 to 60 feet—and usually about the large limbs where the main trunk divided. Sometimes they would cover sections four to six feet long."

Apparently this orchid is fairly common in the Lake Waccamaw section and possibly in other places in the southeastern part of the state, although, so far as known, only two collections exist which are represented by specimens.

It may be of interest to add that collections of *Epidendrum conopseum* have also been made in the neighboring state of South Carolina. Dr. W. C. Coker of the University of North Carolina collected the orchid from a large limb of live oak at Myrtle Beach on July 13, 1932, and H. W. Ravenel collected it along Santee Canal where he said it was growing as a "parasite on *Acer rubrum*." There may possibly be some other collections from South Carolina which have escaped our notice.

DUKE UNIVERSITY,
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PLATE 11



EPIDENDRUM CONOPSEUM AT LAKE WACCAMAW, N. C.

NEW AND LITTLE KNOWN ALGAE FROM NORTH CAROLINA

By L. A. WHITFORD

PLATE 12

For several years the writer has been collecting freshwater algae in the piedmont and coastal plain of North Carolina. Among the more than 360 species and varieties identified from these collections is a number of species either new, or rare and little known. Of the species listed below one is undoubtedly new, two are new to the United States and the others are very rare or have been collected only a few times in this country.

The dimensions are given for each species as well as the months and places of collection. Other states in which the various species have been collected are given. Descriptions or emended descriptions are given for several and six are figured.

The author wishes to acknowledge the helpful criticism of this work by Dr. L. H. Tiffany of Ohio State University.

CHRY SOPHYCEAE

Chrysomonadales

Chrysosphaerella longispina Lauterborn.

Average cells 9μ in diameter, 15μ long, spines $19-39\mu$ long. Colonies $50-250\mu$ in diameter. In North Carolina collections the colonies are small ($47-58\mu$).

From 3 ponds, Cumberland County. May. Also in Wisconsin and New York.

Relatively abundant in Cottonade and Lakewood ponds.

Synura adamsii G. M. Smith.

Cells 7.5μ in diameter, $39-50\mu$ long. Colony $78-93\mu$.

From a swamp and several ponds, Cumberland County. May. Also in Wisconsin and Iowa.

The long narrow cells are loosely joined at the center of the colony and oscillate as the colony rolls slowly over.

CHLOROPHYCEAE

Volvocales

Pandorina charkowiensis Korshik. Fig. 1.

Cells $10\text{--}15\mu$ in diameter. Colony $43\text{--}68\mu \times 58\text{--}88\mu$. The colonies in North Carolina collections average smaller ($50\mu \times 65\mu$).

Plankton, Neuse and Trent Rivers and Core Creek, Craven County. June and September. Also in California.

The ribs on the chloroplast are obscure and most easily seen in freshly collected specimens. Red eye-spots were not observed.

Ulotrichales

Basicladia chelonum (Collins) Hoffm. and Tilden.

Basal cells $12\text{--}35\mu$ in diameter, $680\text{--}1360\mu$ long, other cells $27\text{--}50\mu \times 58\text{--}156\mu$. North Carolina specimens are slightly larger than the average.

Attached to a small turtle, White Lake, Bladen County. May. Also in Michigan, Iowa, Massachusetts, and Ohio.

Upright branches arise from a corralloid basal system. Lower branches remain nearly parallel to the main branch but the occasional upper branches digress at nearly right angles. Several cells were filled with zoospores or gametes and some were escaping through a pore in the wall.

Oedogoniales

Oedogonium nebraskense Ohashi.

Cells $20\text{--}27\mu$ in diameter, $57\text{--}230\mu$ long; oogonia $60\text{--}67\mu \times 70\text{--}79\mu$; oospores $53\text{--}64\mu \times 60\text{--}76\mu$. In North Carolina specimens the oogonium is larger ($74\text{--}78\mu \times 105\text{--}109\mu$) and the oospores average $60\mu \times 70\mu$.

Lake Raleigh, Wake County. April 15, 1935.

This seems to be the first recorded collection of this species in America after it was described from a collection made in Lincoln, Nebraska, April 22, 1919. Note that in spite of the difference in latitude and climate both the collections were made in mid-April

Chlorococcales

Pachycladon umbrinus G. M. Smith.

Cell without processes $8.5\mu \times 15.5\mu$; processes $35\text{--}50\mu$ long.

Apex city reservoir, Wake County. May. Also in New York and Ohio.

The cells were typical but rare in the plankton.

Tetraedron quadricuspidatum (Reinsch) Hansgirg. Fig. 2.

Cell without spines 36μ in diameter, 50μ long. North Carolina specimens average slightly longer and narrower than the type.

Carolina Pines Pond and a small pond near Swift Creek, Wake County. October.

About half the cells observed had only 3 spines but were otherwise typical.

Tetrallantos lagerheimii Teiling.

In the North Carolina collection the cells are 4μ in diameter, 15μ long. Colony 35μ .

Plankton, Cumberland Pond No. 2, Cumberland County. May. Also in Wisconsin and Mississippi.

There is a definite yellowish envelope. The cells are arranged in the characteristic way and attached by cellulose (?) processes.

Siphonales

Vaucheriopsis arrhyncha (Heidinger) Heering. Figs. 3, 4.

Filaments 100μ in diameter, oospores 150μ . In the North Carolina specimens, filaments $87-115\mu$; oogonia $155-164\mu \times 164-170\mu$; antheridia 35μ in diameter 77μ long; pedicels $341-429\mu$ long.

The original description should be emended to read *oil droplets present in filaments and spores*.

Ditch, State College orchard, Wake County. Apr.-May. This appears to be the first collection of this species since it was described from Freiburg, Germany, in 1908.

Tests with the fat stains Sudan III, Scalet R, and with osmic acid show oil droplets to be present in filaments and spores. Pedicels with one oogonium are more abundant than those with two. Sex organs occasionally are produced at the ends of filaments. Otherwise the material seems entirely typical.

Zygnematales

Phymatodocis nordstediana Wolle. Fig. 5.

In the North Carolina collections the cells are $55-62\mu$ in diameter, $43-47\mu$ long.

Dividing cells indicate that the described method of cell division is correct and therefore different from that in *Desmidium*.

From a sand pit, Lillington, Harnett County, in May and Partin's Mill pond, September, 1935. Also in New Jersey.

This rare Desmid has been reported only twice from this country, and not since 1895. It may be less rare than previous collections indicate.

In both collections it was present in the plankton and relatively few plankton collections have been made in the South Atlantic coastal plain.

DINOPHYCEAE

Peridinium wisconsinense Eddy.

Cells 48–56 μ in diameter, 55–64 μ long.

Bladen County. May. Lake Raleigh, Wake County. April and October. Also in Wisconsin and Minnesota.

This species was relatively abundant in the plankton of several ponds.

Ceratium curvirostre Huitfeldt-Kass. Figs. 6 and 7.

(*Peridinium carolinianum* Bailey)

Cells 55–103 μ in diameter at the groove, 156 μ long. North Carolina specimens are 66–78 μ in diameter and 129–154 μ long.

The apical horn is short, curved sharply to the right, and always pointed. There are two antapical horns one longer and stouter than the other. In some cases at least the protoplast escapes from the mother-cell wall before division and each daughter cell develops an entirely new wall. (Fig. 6.)

Plankton of coastal plain ponds. May to October.

The writer agrees with Pascher in regarding the above a valid species and not merely an ecological variation of *C. hirundinella* (O. F. Muller) Schrank. Both species have been collected in abundance in numerous streams and ponds in the lower piedmont and eastern North Carolina. In one pond both were present and there were no intergrading forms. In a number of other cases the two have been collected the same day from different ponds of almost identically the same size and conditions and but a few miles apart. *C. hirundinella* seems to be the form *rubustum*. It sometimes has only two antapical horns but the apical horn is invariably long, straight and truncate. The general appearance is consistently different, as shown by the figures. *C. curvirostre* is rare in the piedmont but *C. hirundinella* has been collected throughout the eastern half of the piedmont and the coastal plain. (Figs. 6, 7, and 8.)

EUGLENOPHYCEAE

Trachelomonas lolliensis sp. nov. Figs. 9 and 10.

Lorica ellipsoidal, converging rather sharply at the anterior end into a long collar and at the opposite end into a posterior process. Surface granulate, studded with stout pointed spines. Color brownish yellow. Collar elongate-cylindric, terminal margin bearing 5 or 6

pointed, divergent spines. One or two lateral spines. Posterior process hollow, nearly identical with collar but slightly conic. Usually with one or two lateral spines. Three or four somewhat divergent spines at end.

Cells without spines $5-11\mu$ in diameter, total length $35-40\mu$. Collar $5-11\mu$ in diameter 12μ long. Posterior process $4-8\mu$ in diameter 12μ long. Body spines $3-5\mu$ long.

Apex city reservoir. May, 1935.

The organism swims with a slow rolling motion.

It was rare at the time collected and was not present in a collection made at the reservoir in October, 1935. Collection of the writer No. 284.

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EXPLANATION OF PLATE 12

All drawings were made with the aid of a camera lucida. Cells in Figs. 6, 7, and 8 drawn in outline only.

FIG. 1. *Pandorina charkowiensis* Korshik. $\times 425$.

FIG. 2. *Tetradron quadricuspidatum* (Reinsch) Hansgirg. $\times 500$.

FIG. 3. *Vaucheriopsis arrhyncha*. (Heidinger) Heering. Fruiting branch with mature oospore. $\times 150$.

FIG. 4. *Vaucheriopsis arrhyncha*. Showing clumping of chloroplasts in antheridium. $\times 150$.

FIG. 5. *Phymatodocis nordstediana* Wolle. Showing cell division and end view of cell with chloroplast. $\times 310$.

FIG. 6. *Ceratium curvirostre*. Daughter cells with complete new walls and part of mother-cell wall. $\times 325$.

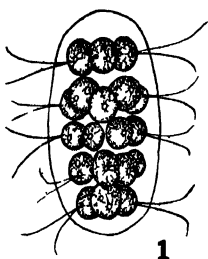
FIG. 7. *Ceratium curvirostre* Huitfeldt-Kass. From Partin's mill pond. $\times 280$.

FIG. 8. *Ceratium hirundinella* (O. F. M.) Schrank. From same collection as Fig. 7. $\times 280$.

FIG. 9. *Trachelomonas lolliensis* sp. nov. $\times 1180$.

FIG. 10. *Trachelomonas lolliensis*. Optical section. $\times 1180$.

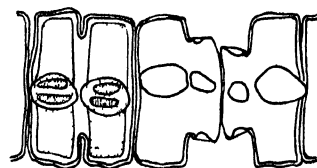
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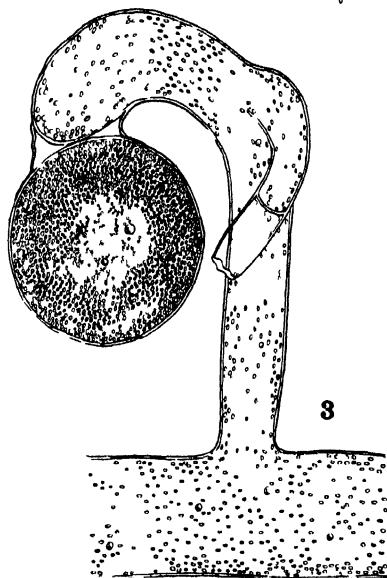
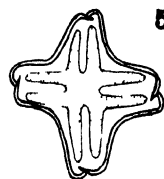
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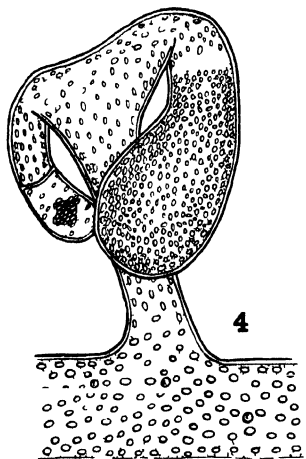
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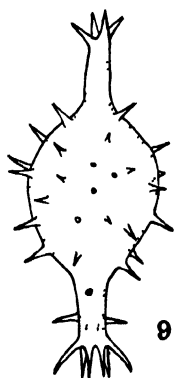
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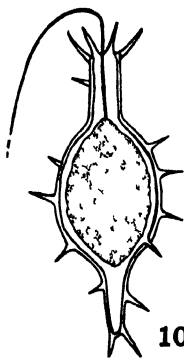
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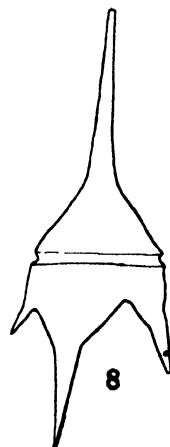
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THE PRODUCTION OF MATURE PERITHECIA OF *CORDYCEPS MILITARIS* (LINN.) LINK IN LABORATORY CULTURE

By LELAND SHANOR

PLATE 13

De Bary (1) in 1867 was able to get the production of perithecia of *Cordyceps militaris* (Linn.) Link in his laboratory, but was never able to get these fruit bodies to produce the mature asci. In only one case ~~was~~ he able to obtain even immature perithecia and these were borne on an abnormal fruit body.

The descriptions and discussions of this fungus by Tulasne (4) and others, as far as I have been able to find, were made from specimens collected in nature. They did not attempt to carry on any culture work. Pettit (3), in 1895, attempted to obtain in culture the production of the perfect stage of this fungus and others of its genus, but without success. Varitchak (5), in 1927, used material collected in nature to carry out his cytological studies.

The authors of the more recent textbooks on mycology make no mention of the work of de Bary on the attempt to obtain the production of mature perithecia of *Cordyceps militaris*. The following report appears, therefore, to be the first to describe the growth of any species of *Cordyceps* from spore to spore in the laboratory.

Specimens of *Cordyceps militaris* were brought into the laboratory by a student on October 10, 1935. These were growing on the pupae of some small Lepidoptera. They were at that time identified and checked with specimens in the herbarium. They were then placed in a petri dish on filter paper and allowed to dry.

Upon finding that this fungus had not been cultured to maturity, an attempt was made to induce the germination of the spores and to get the fungus in culture. A pupa bearing the stromata was placed in a moist chamber and soon normal "puffing" of spores began. To get spores free from contamination, they were taken from the ostioles of the perithecia by touching them with a sterile needle and then transferring them to a petri dish containing a sterile malt extract agar (20 gms. malt extract, 15 gms. agar, 500 c.c. distilled water). This planting

of the spores was done on October 23, and two days later the spores had germinated and had produced minute, white, cottony masses of hyphae that were scarcely visible to the unaided eye. Five days later the colonies were over a centimeter in diameter and now hyphae were cut out and transferred from these to tubes containing some of the same culture media. These cottony cultures remained white for three weeks or a month and then began to change to a lemon color, later becoming a deep orange. In the test tubes where the mycelium pressed against the side of the tubes, it presented a reddish color (fig. 1). This growth of hyphae produced conidiospores abundantly which were borne on elongated, awl-shaped sterigmata. These sterigmata may be either terminal or lateral and when they are lateral may be borne singly or in whorls around the hypha. These conidiophores agree with those described and figured by de Bary (1) and Pettit (3). The conidiospores germinated readily when placed on the malt agar culture media. In places on the surface of the cultures in test tubes the hyphae would sometimes cluster together, rising above the rest of the culture forming a coremium. However, these bore only conidiospores, there being no indication of perithecia-bearing fruitbodies ever produced on this culture media.

On September 28, a larva of the Imperial Moth, *Basilona imperialis* Drury, was collected and brought into the laboratory to metamorphose. It was fed constantly with fresh leaves until the time of pupation on October 12. This pupa was now laid aside in a container on dry filter paper and was still quite lively on November 19, when it was inoculated with hyphae from my cultures. This was done by placing a few of the hyphae into the body of the pupa with a sterile needle. The pupa was now placed in a moist chamber and kept damp with filter paper. On the third and fourth days after this operation the pupa showed less signs of life and was found to be dead on the fifth day. Parts of its body had collapsed, noticeably the wing and antennae cases. The pupa now was converted into a sclerotium by the growth of the fungus. A section of its body later showed that almost all of the soft internal parts had been digested by the fungus.

On December 13, orange colored clusters of hyphae began to push out of the body through the spiracles and at thin places on the abdomen. These continued to grow until, by December 23, they were over a centimeter long. They now ceased to develop further, but produced white, conidia-bearing hyphae. These may have been the Isarial stage, but they do not agree with the description of the *Isaria farinosa* Fr. described by Tulasne (4) as the imperfect stage of *Cordyceps militaris*.

No further development took place until about January 6, 1936, when another knob-like projection made its appearance. By January 13, it had grown to a length of two centimeters and the shape of this body was that of the typical *Cordyceps* fructification. The whole surface of this fruit body was covered smoothly with orange colored hyphae and as yet no indications of the appearance of perithecia were seen. Growth continued, and by January 20, this body had reached a length of over three centimeters. This was somewhat larger than the parent fruit bodies, but the greater amount of nourishment present now might explain this. This pupa was much larger than the one on which the original fructifications were found. Upon examining this fruit body with binoculars, it was found that small dark spots appeared scattered thickly among the hyphae from about midway from its base to its tip. Upon pushing the hyphae back from one of these with a needle, it was found to be the ostiole of a developing perithecium. On January 21, one of these perithecia was removed and examined with the compound microscope. Ascogenous cells were abundant and hook processes were quite numerous. For a couple of days more the development continued and the perithecia became more prominent as the downy hyphae enveloping them collapsed. Development now ceased and over the whole surface of the fruit body downy, white, conidia-bearing hyphae appeared. This suggested that some deficiency in the growing environment preventing further development must exist. Therefore, other experiments were begun.

I had by this time received a supply of the pupae of a common moth, *Callosamia promethia* Drury, from my brother from Butler, Pennsylvania. The following experiments were carried out on these (figs. 2 and 3).

Six pupae were inoculated while still alive, as was done in the first experiment, and then placed in sterile test tubes to be observed until it was certain that they were killed by the fungus. Four were then placed in a sterile container in moist, autoclaved Sphagnum moss. The other two were each placed in a small Erlenmeyer flask and kept moist with filter paper.

Still two other pupae were autoclaved and then inoculated in the same manner as was done with the others. When the fungus had become thoroughly established, one of these was placed in a container in moist, sterile Sphagnum and the other was placed in a small flask to be kept constantly moist with damp filter paper.

All of the cultures made on the living pupae developed at about the same rate as had occurred in the first experiment. Those in the Sphag-

num, however, developed a little faster. Those grown in the flasks ceased development after the production of young perithecia and produced the white, conidia-bearing hyphae as had occurred in the first case. Those cultures in the Sphagnum went on to produce mature perithecia and liberated spores as freely as do fructifications found in nature. This period of development from the inoculation of the pupa until mature fruit bodies had been produced and the "puffing" of the spores was observed varies from 45 to 60 days. After the spores had been mostly liberated, the production of the white, conidia-bearing hyphae took place all over the surface of the fructification when the cultures were kept thoroughly damp.

Those cultures made after the pupae were autoclaved failed to produce anything more than thickly matted clusters of conidia-bearing hyphae. The hyphae in the cultures kept in the Sphagnum remained white, a characteristic found by Pettit (3) to occur in cultures kept in the dark. Those cultures kept in the flask showed a thick mass of oddly-clustered groups of orange colored hyphae (fig. 4). No initials of perithecia-bearing fructifications were noted in either case.

This experiment to secure the development of normal, mature fruit-bodies has been repeated several times, and each time pupae inoculated when living and then placed in the moist, sterile Sphagnum have produced perithecia containing asci with mature spores. When a single fruit body was produced on a pupa, this stroma was considerably larger than any of those produced on a pupa bearing several. The large one may attain a length of 7 cm. and a breadth of 8 mm. The largest of those produced when several arise from a single pupa was $4\frac{1}{2}$ cm. long and 4 mm. wide at its broadest point (figs. 5, 6, and 7). All of these were considerably larger than the parent fruit bodies found growing on a much smaller pupa.

Attempts to obtain the production of perithecia on such media as malt extract agar and media rich in protein content as: fat pork, lean pork, lean beef, egg yolk, egg albumin, or an agar media made from the substance obtained from boiled pupae have all been unsuccessful. The fungus grows quite rapidly on all of these culture media except the fat pork and produces a dense mat of hyphae all over the surface of the media. However, only conidia-bearing hyphae have ever been produced on any of these.

Further experiments are in progress to determine how infection occurs in nature; conditions which control the production of fruit bodies; the host range of the fungus; and also its cytological development.

SUMMARY

1. The mature perithecia of *Cordyceps militaris* (Linn.) Link, borne on normal fruit bodies, were obtained in laboratory culture for the first time.

2. Live Lepidopteran pupae when inoculated and placed in moist, sterile Sphagnum moss have been found to produce normal, mature fruit bodies consistently.

3. Perithecia-bearing stromata of *Cordyceps militaris* are not produced on pupae that have been autoclaved before the inoculations were made, even if placed in moist, sterile Sphagnum.

4. No initials even of the perfect stage of *Cordyceps militaris* have been obtained on various culture media, although those media were rich in protein content.

5. The size of the fruit body varies greatly with the size of the host pupa and with the number of stroma produced on a single pupa.

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Dr. J. N. Couch, under whom this study was made, for his direction and invaluable suggestions during the course of this study. I wish to thank Dr. W. C. Coker for his kindly suggestions and his interest in this study.

I also wish to acknowledge my obligation to my brother, Donald, for sending me a good supply of the pupae of *Callosamia promethia* upon which these experiments were carried out; and to Mr. Don Ritchie for assistance in checking the literature; and to Mr. J. R. Raper for his assistance in the preparation of the photographs.

THE UNIVERSITY OF NORTH CAROLINA,
CHAPEL HILL, N. C.

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EXPLANATION OF PLATE 13

- Fig. 1. The growth of the mycelium of *Cordyceps militaris* produced on malt extract agar in a test tube. $\times 1$
- Fig. 2. Cocoons of the moth *Callosamia promethia* Drury. $\times 1$
- Fig. 3. Pupae of the same removed from the cocoons. $\times 1$
- Fig. 4. The growth produced by the fungus on an autoclaved pupa that was kept constantly moist by damp filter paper in a small Erlenmeyer flask. $\times 1$.
- Fig. 5. A single large fruit body produced on an inoculated live pupa which was buried in moist, sterile Sphagnum. $\times \frac{5}{8}$
- Fig. 6. Three mature stromata produced on a single, inoculated, live pupa which was buried in moist, sterile Sphagnum. $\times 1\frac{1}{4}$
- Fig. 7. The same specimen as in fig. 6 after being removed from the Sphagnum and the culture container. $\times 1$

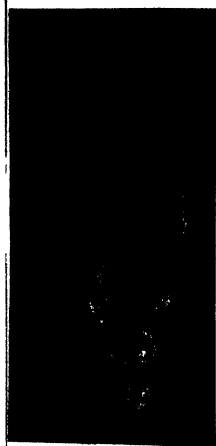
PLATE 13



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4

A NEW AMANITA AND NOTES ON BOLETUS SUBALBELLUS

By H. C. BEARDSLEE

PLATE 14

The genus *Amanita* is so conspicuous and seems so thoroughly studied that the discovery of a species not only new but unique in its characters is a surprising event. This winter, however, in collecting at Altamonte Springs, Florida, we have had this experience.

Early in December a white *Amanita* appeared in sandy soil under pine trees, the appearance of which seemed unusual. The pileus was pure white and glabrous except for occasional closely appressed patches of the volva. The stipe was always deeply buried in the sand and was abruptly enlarged into a bulb which extended below into a well developed, pointed root 5-8 cm. long. This was quite suggestive of forms of *A. solitaria* rather than of *A. verna* with its rounded bulb. From the outer margin of the bulb the remnants of the volva extended in a loose cylinder 2-4 cm. long, not at all like the free volva of *A. verna*. The stipe itself was firm and solid and at times at least when sectioned had a distinct odor of chlorine. The solid stipe, chlorine odor, and pointed root thus seemed to suggest the *solitaria* group, while the loose volva, though unusual in its extended cylindrical form suggested rather the *phalloides* group.

The spores when examined proved to be most unusual. All of the *Amanitas* have spores which are either globose or very broadly ellipsoid. This species has spores as much as 15μ long and barely $4-4.5\mu$ in diameter, being so narrowly cylindrical that they resemble nothing so much as an ordinary sausage. No *Amanita* known to me has spores at all similar. It has seemed best to describe this species as new, using the specific name descriptive of its spore form.

***Amanita cylindrispora* sp. nov.**

Pileus 4-8 cm. latus, hemisphericus, demum expansus, albus, viscidus, glaber aut cum volvae fragmentis appressis, epiderme firmo, separabili. Lamellae albae, confertae, adnatae. Stipes 6-9 cm. longus, 8-10 cm. crassus, abrupte bulbosus, radice acuto 5-7 cm. longo prolongatus. Sporae cylindricae, $10-15 \times 4-4.5\mu$. Odor chlorinatus.

In pinetis, Altamonte Springs, Florida. Dec. 1935.

Boletus subalbellus Murrill

Pileus up to 7 cm. broad, convex, rather firm, white, becoming isabelline, minutely appressed tomentose, margin thin, at first incurved; flesh white, not changing color.

Tubes white, mouths small, round, 10–14 to a centimeter (0.7–1 mm. in diameter), almost free, 5 mm. long at center.

Stipe 6–8 x 1.5–2.5 cm., stuffed, soon hollow and fragile, colored like the pileus.

Spores 10–14 x 5–6 μ , hyaline under the microscope. (Caps set for spores but no good prints obtained.)

This is frequently found in lawns and open woods at Altamonte Springs. The appearance is that of a white *B. castaneus*. The spores of my plants do not coincide with Murrill's figures, but Dr. Walter Snell says they agree with the spores of the type.

ALTAMONTE SPRINGS, FLA.

PLATE 14



AMANITA CYLINDRISTORA

THE PSAMMOCHARIDAE OR SPIDER WASPS OF NORTH CAROLINA

By C. S. BRIMLEY

THREE TEXT FIGURES

The Psammocharids are vespoid wasps distinguished from related families by having the posterior angles of the pronotum prolonged backward to and beneath the tegulae, by having the first two abdominal segments not separated from each other by a constriction, and by the possession of elongate legs, the hind femora always extending beyond the middle of the abdomen, and in addition they are always smooth, never roughly sculptured.

Our species range in size from very small wasps, only 5 mm. in length to very large ones, 25 mm. long, and still larger forms occur in the southwestern United States. In color more than half our species are black or blackish without any markings whatsoever; while more than half the rest possess only restricted markings, a few only being entirely reddish or with extensive reddish or yellowish markings.

Generic and specific distinctions are based largely on the female sex, hence it is sometimes not easy to place a male with absolute certainty. In some species the sexes are alike and easily correlated; in others the opposite is the case.

The Psammocharids are active wasps and the larger species are common on flowers, having in common with many other kinds of wasps a special liking for those of Snow-on-the-Mountain (*Euphorbia marginata*). The smaller species are mostly taken by sweeping and seem less plentiful than the larger kinds, in contradiction to the usual rule among insects.

The various species dig subterranean nests which they provision with spiders as food for their grubs; those of the genus *Ceropales* however are parasitic on other members of the family.

An even one hundred species have been recorded from the state. Some of them however are doubtless the two sexes of species in which the sexes have not yet been correlated. This is however offset by the fact that in only one locality, Raleigh, has there been at all thorough collecting done in the family, and quite a number of other species may yet be expected from the state.

I have consulted all available literature for which see bibliography at the end of the paper, but I have found the publications on this group by Dr. Nathan Banks of the greatest help to me. Dr. Banks has also determined a number of species for us.

It should be borne in mind that all generalised statements apply only to the forms under consideration and not necessarily to extralimital species.

Keys to subfamilies and tribes, as well as to all genera and species found in the state follow and are accompanied by brief descriptions. I have seen all the species included except the following: *Pseudagenia mellipes interior* Banks, *Pseudagenia nigrella* Banks, *Episyrus posterus* Fox, *Lophopompilus ilione* Banks, *Lophopompilus bengtssoni* Regan, *Pompiloides minora* Banks, and *Psammochares gracilicornis* Banks.

Of the technical terms used in this paper, most refer to the wing cells and wing veins and will be sufficiently elucidated by the figures of the three Psammocharid wings illustrated. These figures were drawn from specimens in the Collection of the Division of Entomology by Mr. D. L. Wray, a coworker in this division to whom I am greatly indebted for them.

A few other terms may need explanation. These are:

Comb. In certain females of the genus *Psammochares* there is a series of long spines on the outer side of the front tarsus and these are termed a "comb." In *P. fabricii* for instance there are four on the first joint (metatarsus), two on the second, and one on the third, all of nearly equal length.

Petiolate. When said of the second submarginal it means that the veins on each side of that cell coalesce above, so that the first and third submarginals are in contact above it.

Postscutellum. The small transverse piece between the scutellum and propodeum. It is now considered to be the metanotum or last segment of the thorax, while the propodeum, the apparent last segment of the thorax is now considered to be morphologically the first segment of the abdomen. As the propodeum used to be called metanotum by the older authors the use of that name is somewhat confusing and I prefer to use postscutellum.

KEY TO THE GROUPS OF PSAMMOCHARIDAE FOUND IN NORTH CAROLINA

1. Claws of hind tarsi close together and bent at right angles near the middle; labrum always visible Subfamily CEROPALINAE
- Claws of hind tarsi divergent, not bent at right angles; labrum usually not exposed Subfamily PSAMMOCHARINAE. 2

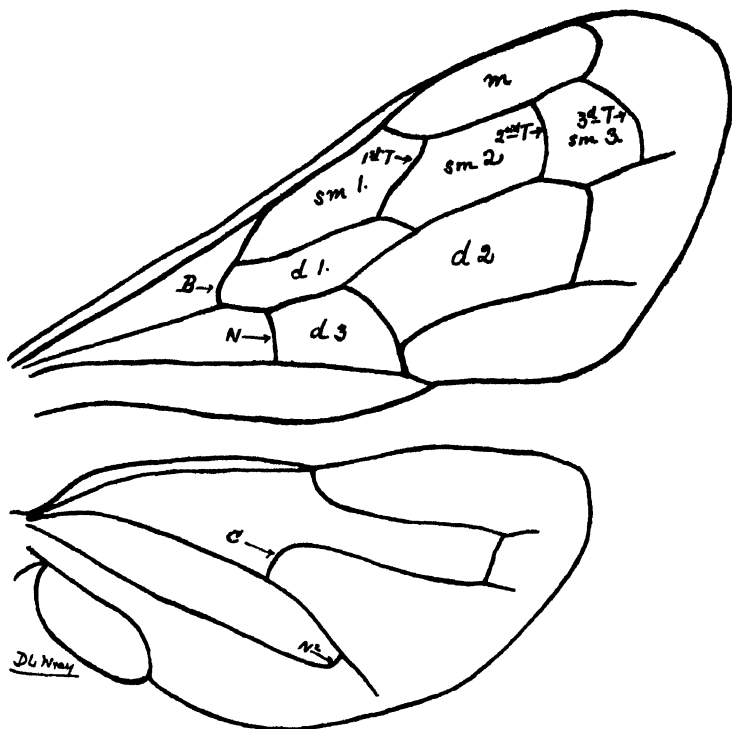


Figure 1. Wings of *Pepsis elegans*. Note three submarginal cells in front wing, no pocket at base of second discoidal cell, and that the nervulus and nervellus are well beyond the basal veins of their respective wings.

Lettering of figures

- A. Cells. m. Marginal cell in front wings. (R1)
 sm1, sm2, sm3. = First, second and third submarginal or cubital cells in front wings. (R. R5. R4)
 d1, d2, d3. = First, second and third discoidal cells. (M4, 1st M2, M3)
- B. Veins. B. = Basal vein in forewing. (m-cu)
 N. = Nervulus in forewing. (M4 \times Cu1)
 R1. = First recurrent vein in forewings. (M3 \times 4)
 R2. = Second recurrent vein in forewings. (M2)
 1st T, 2d T, 3d T. = First, second and third transverse cubitals in forewings. (R5 \times rm, R5, R4)
 C. = Cubitus or basal vein in hind wings. (m-cu)
 N1. = Nervellus in hind wing. (M3)

Note also that the vein below the submarginal cells is known as the *cubital vein*, and that between the submarginals and the marginal cell as the *radial vein*.

The Comstock equivalents of the cells and veins are given in parentheses.

2. Second discoidal cell without a pocket at its base; second ventral segment with a transverse furrow (absent in some males) Tribe PEPSINI
 Second discoidal cell with a small pocket at its base; second ventral without a transverse furrow Tribe PSAMMOCHARINI

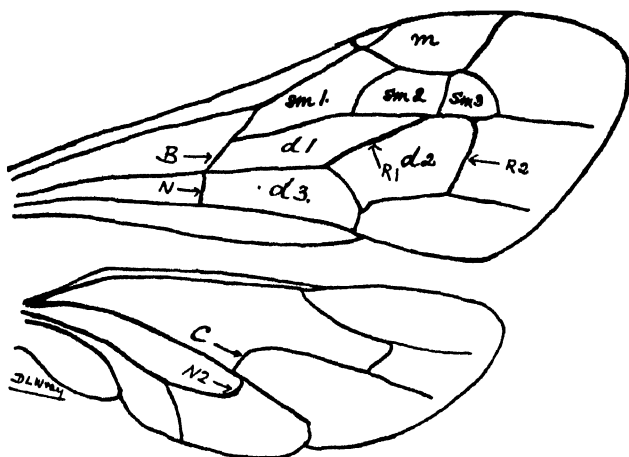


Figure 2. Wings of *Psammochares philadelphicus*. Note three submarginals, and and small pocket at base of second discoidal in front wings; nervulus interstitial with basal vein in forewings, nervellus beyond cubitus in hind wings.

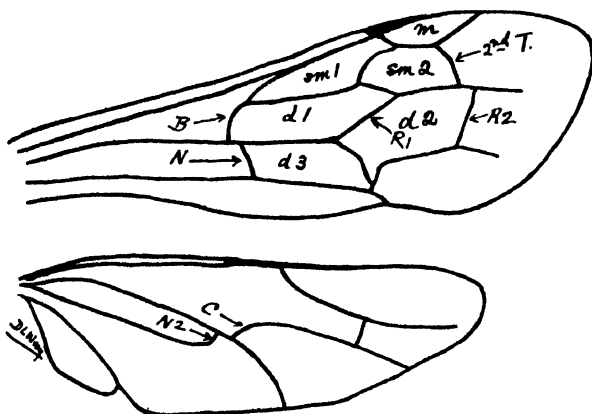


Figure 3. Wings of *Planiceps niger*. Note only two submarginals in front wings and nervellus before cubitus in hind wings.

Subfamily Ceropalinae

These wasps are parasitic in the nests of other species of the family. Only the type genus is found in North Carolina.

CEROPALES Latreille

The antenna is never convolute in either sex, and the body always has some pale markings. Six forms have been taken in North Carolina.

KEY TO THE NORTH CAROLINA SPECIES OF CEROPALES

1. Wings and abdomen wholly black; length about 10 mm. 2
 Wings hyaline or nearly so, abdomen with pale markings; smaller species . . 3
2. Legs mainly black but with the hind femora rufous *bipunctatus* Say
 Legs beyond coxae entirely rufous. *tibialis* Banks
3. Antennae longer than head and thorax; abdomen with complete even crossbands on all or most of the segments; legs black and yellow
 fraterna Smith
 Antennae not longer than head and thorax; crossbands on abdomen interrupted or if complete, not even. 4
4. Length about 7 mm. Crossbands of abdomen narrowly interrupted in middle; legs yellow, hind tarsi with each joint narrowly black at base
 longipes Smith
 Length 5 mm. Crossbands widely separated in middle, hind tarsi plain dusky. 5
5. Femora rufous *foxii* Aldrich
 Femora black. *hatoda* Brimley

C. bipunctata Say. Length 10 mm., spread 18 mm.; abdomen usually curved under. Wings black, body black; anterior orbits white or yellowish on median half; a yellowish spot above hind coxae; hind femora rufous except at base and extreme apex. Males have in addition face below and between antennae, clypeus, labrum, underside of scape and of second antennal segment and a transverse band near apex of pronotum, white. One male has apex of front femora and whole of front tibiae rufous internally. Raleigh, Winston-Salem, Old Fort, Sept., Oct.; Bryson City, late August.

C. bipunctata tibialis Banks. Like preceding but legs wholly rufous. Greensboro, Raleigh, mid July, two males. Type locality Southern Pines, N. C.

C. fraterna Smith. Length 6-7. Female, face yellow with vertical black stripe below antennae, pronotum edged behind with yellow, a yellow spot each side on collar and another above hind coxae; broad yellow band across first abdominal segment and others at apex of remaining segments; scutellum and postscutellum with yellow spot and sometimes a yellow spot above mid coxae; femora mostly black, coxae black and yellow, rest of legs mainly yellow. Male similar but no black below antennae and band on first abdominal segment is divided into two broad yellow spots. Raleigh, April, May, Sept., Oct.; Laurinburg, Oct.; Highlands, Sept.; Linville Falls, June; Marion, Aug.

C. foxii Aldrich. Markings except on abdomen much as in *fraterna* but less prominent; each abdominal segment with a narrow whitish mark on each side near apex widely separated from its fellow on the other side, last segment white; legs beyond coxae rufous except hind tibiae and tarsi. Length 5. Raleigh, May, Sept., male and female.

C. hatoda Brimley. Hind legs and all femora black, otherwise as in *foxii*. Length 5. Raleigh, Sept., one male.

C. longipes Smith. Legs red except hind tarsi, the joints of which are very narrowly black at their extreme bases; pale bands on abdomen notched in front on each side, that on second segment barely interrupted. Length 6. Raleigh, June, one male.

Sub-family **Psammocharinae**

Tribe **PEPSINI**

Distinguished by the characters given in the key and in addition the females of the genera *Priocnemis*, *Cryptocheilus*, and *Pepsis* have the hind tibiae serrate behind, that is with a series of small projections each of which bears a spine. This feature however is absent or poorly developed in the males of those genera and absent in both sexes of the other genera.

KEY TO NORTH CAROLINA GENERA OF PEPSINI

1. Hind tibiae not serrate behind and without spines or with only feeble ones; nervellus not beyond cubitus in hind wing..... 2
 Hind tibiae serrate behind and with well developed spines in the females, usually with only spines in the males..... 3
2. Propodeum with erect hair above **PSEUDAGENIA** Kohl
 Propodeum without erect hair above..... **AGENIELLA** Banks
3. Last joint of hind tarsi without spines beneath or with only a few weak ones
 **PRIOCNEMIS** Schiodte
 Last joint of hind tarsi spined beneath, nervellus usually beyond cubitus; larger species with black wings..... 4
4. Second cubital cell usually shorter than third, receiving first recurrent vein at its middle or beyond..... **CRYPTOCHEILUS** Panzer
 Second cubital much longer than third, receiving first recurrent near its base
 **PEPSIS** Fabricius

AGENIELLA Banks

Small Pepsines with the hind tibiae smooth and usually without spines; propodeum without erect hair above. Ten species are on record from the state, two of which are known only in the female sex and eight only as males. The male of *Priocnemis pompilus* is included in the

key as it is practically indistinguishable from an *Ageniella* and some of the other males may also be the males of small species of *Priocnemis*.

KEY TO NORTH CAROLINA SPECIES OF AGENIELLA

1. Females with six visible abdominal segments and twelve antennal joints 2
Males with seven abdominal and thirteen antennal segments 3
 2. Wings uniformly blackish, body wholly black *bombycina* Cr.
Wings hyaline cross banded with dusky, body wholly reddish. *accepta* Cr.
 3. Abdomen with red on first two or three segments 4
Abdomen wholly black 6
 4. Legs except hind tibiae and four hind tarsi, yellowish red *julia* Brimley
Legs black 5
 5. Tibial spurs white *birkmanni* Bks.
Tibial spurs black *longa* Cr.
 6. Legs partly reddish 7
Legs wholly black 9
 7. Pronotum edged behind with white; all the legs largely red; tibial spurs white *calcarata* Cr
Pronotum all black; red confined mainly to hind femora; tibial spurs black 8
 8. Wings uniformly but faintly dusky, abdomen without a white spot at tip *aludra* Brimley
Wings hyaline with a sharply defined dusky tip *adara* Brimley
 9. Spurs all dark; third submarginal cell higher than wide; wings not darker at tip *Priocnemis pompius* Cresson
At least the spurs of four anterior tibiae white; third submarginal at least as long as high; wings with definite darker tips 10
 10. All the spurs white *subra* Brimley
Spurs of posterior tibiae dark *mintaka* Brimley
- A. *accepta* Cr. Length 9. Raleigh, Kinston, mid June to September, on flowers especially those of *Euphorbia marginatum*. In spite of its wings it looks and acts much like a big red ant.
- A. *adara* Brimley. Length 5. Third submarginal cell longer than high and larger than second; amount of red on hind femora very variable; front tibiae reddish. Raleigh, July to September.
- A. *aludra* Brimley. Similar to preceding but third submarginal higher than long and smaller than second; front tibiae black. Raleigh, May, August, September.
- A. *birkmanni* Bks. Length 5. First two or three abdominal segments red, tibial spurs white, otherwise black. Raleigh, July, August.
- A. *bombycina* Cr. Length 10. Raleigh, May, August, November; Liberty, August. Has been bred from mud nest under bark of pine stump. This species has some spines under last joint of hind tarsi and some on hind tibiae and has been placed by Banks (1933) in a new genus *Phanagenia* Banks, type *P. osceola* Bks., Florida.

- A. calcarata* Cr. Length 5. Clypeus white with square black spot at base. Raleigh, April to September. Possibly the male of *Priocnemis alienatus*.
- A. julia* Brimley. Length 5. Besides characters given in key, it has the face and clypeus wholly black and the wings hyaline faintly tipped with dusky. Raleigh, June, one male.
- A. longa* Cr. Similar to *birkmanni* but the spurs are black. Raleigh, Rocky Mount, May to September.
- A. mintaka* Brimley. Length 5. Spurs of hind tibiae dark; in the type and only specimen the outer side of the third submarginal cell is angulated outwardly and with a short external spur. Raleigh, June.
- A. subra* Brimley. Similar to preceding, spurs of hind tibiae white: outer edge of third submarginal normal. Raleigh, September, two males

PSEUDAGENIA Kohl

Similar to *Ageniella* but the propodeum has erect hairs on the upper surface as well as pubescence.

We have eleven species on record from the state, three represented only by males, four by females and three by both sexes and one of which I do not know the sex.

KEY TO NORTH CAROLINA SPECIES OF PSEUDAGENIA

- | | | |
|--|-------------------------------|----|
| 1 First two or three abdominal segments reddish | <i>marionae</i> Brim. | |
| Abdomen wholly black | | 2 |
| 2. All three pairs of legs mainly yellowish | | 3 |
| Legs mainly or wholly black | | 7 |
| 3. A black line on the inner side of each hind tibia | <i>mellipes interior</i> Bks | |
| Legs wholly yellow | | 4 |
| 4. Females with 12 antennal joints and 6 abdominal segments | | 5 |
| Males with 13 antennal joints and 7 abdominal segments | | 6 |
| 5 All coxae black | <i>mellipes</i> Say | |
| Four posterior coxae reddish yellow | <i>mellipes adjuncta</i> Bks | |
| 6 Hind coxae black, rest reddish | <i>mellipes</i> Say. | |
| All coxae reddish yellow | <i>mellipes adjuncta</i> Bks. | |
| 7 Females with 12 antennal joints and 6 abdominal segments | | 8 |
| Males with 13 antennal joints and 7 abdominal segments | | 12 |
| 8. Femora largely red; coxae and four posterior tibiae and tarsi black | <i>caerulescens</i> Dahlb. | |
| Legs wholly dark | | 9 |
| 9. Front wings with a large dusky cloud on marginal, second and third sub-marginals and below them | <i>pulchripennis</i> Cr. | |
| Wings wholly hyaline | | 10 |

10. Head and thorax black; pronotum angulate behind. *nigrella* Bks.
Head and thorax blue or bluish 11
 11. Larger with broader face; pronotum angulate behind. *architecta* Say
Smaller with narrower face; pronotum arcuate behind *nanella* Bks.
 12. Front tibiae reddish, legs otherwise dark; hair of head largely white
najacra Brimley
Legs wholly black 13
 13. Hair of head mainly black *mariva* Brimley
Hair of head mainly white. *nanella* Bks.
- P. architecta* Say. Length 7-9. Body blue with whitish hair; both second and third submarginal cells longer than high; Raleigh, May, July, September to November; Swannanoa, October, all females.
- P. caerulea* Dahlb. Length 6. Fayetteville, June.
- P. mariva* Brimley. Length 10. Black, shining. Linville Falls, June, one male.
- P. marionae* Brimley. Length 7. First two or three abdominal segments largely reddish; face, scape beneath, and posterior edge pronotum whitish; flagel black. Raleigh, May, three males.
- P. mellipes* Say. Length 7-9. Tibial spurs usually darker than tibiae. Raleigh, Elizabeth City, Statesville, April to September, females; Mt. Mitchell, June, male.
- P. mellipes adjuncta* Bks. Tibial spurs light red like the tibiae; Raleigh, May to August. The male of this and the preceding have the face below and beside the antennae yellowish white and the antennae yellowish below.
- P. mellipes interior* Bks. Southern Pines (type locality). According to the original description the antennae are yellowish and the specimen was therefore presumably a male. I have not seen it.
- P. najacra* Brimley. Length 7. Front tibiae yellowish on inside and at apex, mid and hind tibial spurs reddish. Raleigh, June, male. Hickory, August, male.
- P. nanella* Bks. Length 6. Raleigh, May, June.
- P. nigrella* Bks. Black Mountain, May (Banks). I have not seen it.
- P. pulchripennis* Cr. Length 9. Hind tibiae with short, stout, spines. Raleigh, June, one female.

PRIOCNEMIS Schiodte

Hind tibiae serrate in female, usually not so in male; last joint of hind tarsi with few or no spines beneath. The males of the smaller species are often hard to distinguish from those of the preceding two genera.

KEY TO NORTH CAROLINA SPECIES OF *PRIOCNEMIS*

- | | | |
|----|--|------------------------|
| 1 | Nervellus beyond cubitus, size small | 2 |
| | Nervellus before or interstitial with cubitus | 4 |
| 2 | Body and legs wholly black; female | <i>nebulosus</i> Dahlb |
| | Front and mid tarsi mostly whitish, the joints with narrow black tips; face mostly white; males | 3 |
| 3 | Femora black | <i>pulchrina</i> Cr |
| | Femora rufous | <i>lebyn</i> Brimley |
| 4 | Abdomen with more or less red | 5 |
| | Abdomen wholly black | 9 |
| 5 | Size large, length 12-18; wings black | 6 |
| | Size small, length 10 or under; wings hyaline or banded | 7 |
| 6 | Antennae largely reddish below; hairs on hind tibiae about as long as the spines | <i>validus</i> Cr |
| | Antennae all black; hairs on hind tibiae much shorter than the spines | <i>gomelza</i> Brimley |
| 7 | Abdomen black at tip; wings banded, legs largely red | <i>alienatus</i> Smith |
| | Abdomen wholly red; wings hyaline; legs black | 8 |
| 8 | Pronotum arcuate behind; tibial spurs pale | <i>arcuata</i> Bks |
| | Pronotum angulate behind; tibial spurs dark | <i>directa</i> Bks |
| 9 | Wings hyaline at base, the dusky tip and a dusky band across the submarginals enclosing a subapical hyaline space | <i>germana</i> Cr |
| | Wings not as above, practically unmarked | 10 |
| 10 | Propodeum transversely striate behind; wings black | <i>fortis</i> Cr |
| | Propodeum not striate behind | 11 |
| 11 | Small species, length about 7 mm | 12 |
| | Larger species, length about 10 mm | 13 |
| 12 | Wings uniformly dusky, third submarginal cell about as high as wide | <i>pompilus</i> Cr |
| | Wings hyaline, dusky at tip of front wings; third submarginal decidedly longer than high | <i>agenoides</i> Fox |
| 13 | Face with golden pile; wings hyaline tipped with dusky; abdomen subpetiolate | <i>fulgifrons</i> Cr |
| | Face with black pile, wings more or less dusky with darker centers to the second and third submarginals and second discoidal | 14 |
| 14 | Hind tibiae with about 9 strong teeth, the spines as long as the spaces between them | <i>conicus</i> Say |
| | Hind tibiae with about 6 to 8 weaker teeth, the spines shorter than the spaces between them | <i>minorata</i> Bks |

P. agenoides Fox Length about 7 Raleigh, June, August, September, three females

P. alienatus Smith. Length 8. Wings with large subapical cloud and tip dusky Raleigh, May, June, September; Sunburst, Linville, May; Balsam, September.

P. arcuatus Bks. Length 8. Second recurrent bent rather strongly inward before tip. Raleigh, June, one female.

- P. conicus* Say. Length 10-12. Wings dusky, Raleigh, February to June.
- P. directa* Bks. Length 8. Raleigh, June to September, females only.
- P. fortis* Cr. Length 12-13. Boone, Grandfather Mt., Highlands, September, three females.
- P. fulgifrons* Cr. Length 10. Black with much golden pile, especially on face, under side of prothorax and front coxae. Raleigh, August, one female.
- P. germana* Cr. Length 10. Highlands, July, one female.
- P. gomelza* Brimley. Length 12 (abdomen curved under). First three abdominal segments rufous above. Raleigh, April, two females.
- P. leiby* Brimley. Length 7. Front coxae white anteriorly; tibiae and femora reddish; a faint cloud in second submarginal and below it. Raleigh, Tarboro, June, two males.
- P. minorata* Bks. Length 8. Marion, April; Spruce, May.
- P. nebulosus* Dahlb. Length 10. Black with dusky wings, an evident darker cloud in the second submarginal and below it, and the tip of the wing darker. Raleigh, one female.
- P. pompilus* Cr. Length 5-7. Raleigh, Trenton, June to October, both sexes.
- P. pulchrina* Cr. Much like *leiby* but the legs are without red. Raleigh, June to August, males only. In all probability the male of *nebulosus*.
- P. validus* Cr. Length 19. The first three abdominal segments rufous above. Raleigh, June, July; Wilkes County, July, three females.

CRYPTOCHEILUS Panzer

Large Pepsines with the last joint of the hind tarsi furnished with spines beneath, and usually with the nervellus beyond the cubitus in the hind wings. The body is entirely black in all our species.

KEY TO NORTH CAROLINA SPECIES OF CRYPTOCHILUS

1. Antennae mostly yellow or orange..... 2
 Antennae wholly black..... 4
2. Wings with a rather large yellow spot centering on second and third submarginal cells..... *unifasciatus* Say
 Wings wholly black..... 3
3. Third ventral with a pair of humps beneath; antennae light yellow
 *fulvicornis* Gm.
 Third ventral without humps; antennae dark yellow..... *magnus* Cr.

4. Front wings with a large sharply defined yellow spot on outer third
maculipennis Sm.
 Front wings and body wholly black: marginal cell rounded at apex
idoneus Bks.
- C. fulvicornis* Cr. Length, male 15-17, female 20. Raleigh, Oriental, Beaufort, Overhills, Hobucken, Kingsboro, Statesville, Winston-Salem, Marion, Judson, Cruso, Salisbury, June to October.
- C. idoneus* Bks. Wholly black, including wings and antennae. Length 12-15. Raleigh, Southern Pines, Laurel Hill, Winston-Salem, Marston, June to August, October.
- C. maculipennis* Sm. Length 23. Raleigh, July; Southern Pines, June, two females.
- C. magnus* Cr. Length, male 19. Judson, July; Southern Pines, Wilmington, Beaufort, June to August. I have seen only males.
- C. unifasciatus* Say. Length, male 13, female 20. Raleigh, Elizabeth City, Fayetteville, Swannanoa, Blantyre, Bryson City, Asheville, and Wilkes Co., July to September, both sexes.

PEPSIS Fabricius

Second submarginal longer than third and receiving first recurrent near its base. Only one species occurs in North Carolina but this is the largest member of the family and much larger forms occur in the southwestern United States.

P. elegans Lep. Wholly black with orange antennae. Length about 25 mm, spread about 40. Raleigh, Wilmington, Durham, Southport, Bryson City, Homestead, Lyons, Blowing Rock, Asheville, Wilkes County, Fayetteville, June to September.

Tribe PSAMMOCHARINI

Includes species with a small pocket at base of second discoidal cell and with the claws of the hind tarsi curved and divergent. There is no transverse groove on the second ventral and the hind tibiae are never serrate. More than half our species belong here.

KEY TO GENERA OF NORTH CAROLINA PSAMMOCHARINI

1. Pronotum long, longer than the mesonotum and much flattened above, these characters much less developed in the males. 2
 Pronotum shorter than the mesonotum, arched in profile. 3
2. Three submarginal cells. PEDINASPIS Kohl
 Two submarginal cells. PLANICEPS Latreille
3. Two submarginal cells. APORINELLUS Banks
 Three submarginal cells. 4

4. Basal segment of abdomen with appressed pubescence unlike that on the following segments; claws cleft in both sexes EPISYRON Schiodte
 Basal segment without peculiar pubescence 5
5. Labrum visible, claws cleft in both sexes, antennae of male crenulate, a distinct malar space, body, legs, and wings wholly black ALLOCYTHONYX Ashmead
 Without above combination of characters 6
6. Whole body and legs reddish or conspicuously marked with red or yellow; an impressed median longitudinal line on pronotum behind 7
 At least head and prothorax black except sometimes a white posterior margin to pronotum; no such impressed line on pronotum PSAMMOCHARIS Latreille
7. Upper margin of clypeus even; whole body and legs reddish ARACHNOPROCTONUS Ashmead
 Upper margin of clypeus notched on each side; body with yellow markings; labrum exposed; antennae of male crenulate below .BATAZONUS Ashmead

PEDINASPIS Kohl.

Species with long flattened pronotum and three submarginal cells. The females are very distinct but the males have the pronotum shorter and less flattened and look much like the males of *Epsyron* but may be distinguished by the slightly longer pronotum, the shorter joints of the tarsi, and the unequally cleft tarsal claws

KEY TO NORTH CAROLINA PEDINASPIS

1. Females; wings blackish or banded with dusky; abdomen not wholly black 2
 Males; wings hyaline tipped with dusky; whole body black without markings but with appressed silvery pubescence 4
2. Wings hyaline with dusky cross bands; head and most of body red; length 11 legatus Cr.
 Wings black; length 20-25 3
3. Thorax and abdomen mostly red sanguineus Cr.
 Red confined to upper surface of second abdominal segment brimleyi Malloch
4. With much long erect hair on head and body; second submarginal receiving first recurrent much beyond middle; length 11 brimleyi Malloch
 Without long erect hair on head and body; second submarginal receiving first recurrent in middle 5
5. Tibial spurs black; length about 10 magnus Bks.
 Tibial spurs white; length about 6 legatus Cr.
- P. brimleyi* Mall Black with large orange red blotch on dorsum of second abdominal segment, abdomen compressed on apical portion, second submarginal cell one and a half times as long as third or longer, narrowed more than one half above, third much shorter, about as high as long Raleigh, Marion, Cary, Wilkes County, July to September, mostly on flowers of *Euphorbia mar-*

ginata. What I take to be the male is wholly black with much silvery sericeous pubescence and in addition with abundant long erect hair on head, body, coxae, and hind femora beneath; wings hyaline tipped with dusky. Raleigh, Beaufort, August, three males.

P. legatus Cr. The females have the body mostly red; second abdominal segment black at apex, third and fourth black at apex, white at base, fifth and sixth black; wing with outer third dusky and a dusky band across basal transverse veins, rest of wings mostly hyaline; second and third submarginals short, as high as or higher than long; legs red. Raleigh, Benson, Southern Pines, May, August. What appears to be the male has similar venation and is wholly black except for the white tibial spurs. Raleigh, June, three. The female has the habits of a female Mutillid and does not seem to fly although provided with wings.

P. magnus Bks. Much like the male of *brimleyi* but lacks the erect hair on the body; the second and third submarginal cells are about equal in size. Raleigh, June, two males.

P. sanguineus Cr. Pronotum and mesonotum orange, propodeum dark red; first abdominal segment dark red, second yellow, third yellow, black at apex, fourth to sixth black, the last two whitish at apex; head, both scutels, pleura and legs black; both second and third submarginals longer than high, subequal, second much, third little narrowed above. Raleigh, August, one female on flowers of *Euphorbia marginata*.

PLANICEPS Latreille

Similar to *Pedinaspis* but with only two submarginal cells in the front wings, the second recurrent meeting the cubital vein beyond the second submarginal, the third transverse cubital wanting.

P. niger Cr. Wholly black, wings of the female blackish, those of the male hyaline tipped with dusky. Length females 11-13, males, 5-8. Raleigh, Swannanoa, late May to early October. One male has only a single submarginal cell.

APORINELLUS Banks

With two submarginal cells in the front wings; pronotum short and arched in profile and both recurrents received by the second submarginal cell; propodeum with strong posterolateral angles.

A. fasciatus Smith. Mostly gray; pronotum with a subapical black

band, abdominal segments 2-4 black at base, gray at apex, wings hyaline with dusky tips. Length 6. Raleigh, Aberdeen, Carolina Beach, Marston, June, August, September, both sexes.

EPISYRON Schiodte

Medium sized or small species in which the first abdominal segment is covered with greasy looking prostrate pubescence different from that on any of the following segments; claws cleft in both sexes. Most species have a pair of pale spots at the base of the third abdominal segment which may be partly or wholly concealed when the segment is retracted.

KEY TO NORTH CAROLINA EPISYRON

1. Hind femora and tibiae rufous. *posterus* Fox
Legs without any rufous. 2
 2. Spurs white; hind tibiae with a white stripe at base above; hind margin of pronotum usually white. *snowi* Vier.
Tibial spurs black. 3
 3. Smaller, length 7-8; body and legs wholly black *maneei* Bks.
Larger, length 10-13; pronotum usually white-margined behind; and third abdominal segment with a pair of white spots near base; a white stripe near base of hind tibiae in male. 4
 4. Wings hyaline or subhyaline tipped with dusky; a pair of pale spots at base of second as well as at base of third abdominal segment; front metatarsus of female with four long spines. *quinquenotatus* Say
Wings darker, often uniformly dusky; no pale spots on second abdominal segment; only three long spines on front metatarsus of female. . . *biguttatus* Fabr.
- E. biguttatus* Fab. Variable in size and in presence or absence of white posterior border to pronotum and also in color of wings but all females have only three spines on front metatarsus and none have spots on second abdominal segment. Raleigh, Southern Pines, Elizabeth City, Wadesboro, Aberdeen, Kittrell, Swannanoa, and Bryson City, July to October, only females seen.
- E. maneei* Bks. Raleigh, June, August; Southern Pines, August; Fayetteville, May, all females, possibly the female of *snowi*.
- E. posterus* Fox. Length 7. Distinguished by the red hind legs. Beaufort, summer, 1909, one taken by Mr. Sherman, not now in our collection.
- E. quinquenotatus* Say. Black Mountain, mid August, two females. Two males from Bryson City in July, and Black Mountain, late May may be this or *biguttatus* as they have no spots on the second abdominal segment. Raleigh, August, one male.

E. snowi Viereck. Length 6-8. Distinguished by the white tibial spurs. Raleigh, May, June, August, September. Have seen only males.

ALLOCYPHONYX Ashmead

Wholly black species with a distinct space between the lower end of the eyes and the mandibles, the claws cleft in both sexes and the male antennae crenulate.

We have only a single species.

A. maurus Cresson. Length, males 10-15, females 15-18. Recorded from 14 localities within the state scattered from New Hanover County in the east to Buncombe County in the west and with a seasonal range from May to November. Banks described the male as *A. harpalyce* distinguishing it from the male of *maurus* by the possession of abundant silvery pile on the propodeum and both sexes by having the basal veins dislocated in the hind wings. However, all our males have the silvery pile on the propodeum and both sexes usually have the basal veins interstitial in the hind wings, consequently it seems best to consider *harpalyce* as merely a southern form of the male of *maurus*.

PSAMMOCHARES Latreille

Contains all our species of the tribe Psammocharini without sufficiently outstanding characters to be placed elsewhere.

This genus is divided into a number of subgenera any or all of which are liable in the future to be considered full genera.

KEY TO OUR SUBGENERA OF PSAMMOCHARES

1. Clypeus emarginate in front, much less so in males. 2
 Clypeus not or barely emarginate in front. 3
2. Pronotum angulate behind; propodeum with but little hair above
 NOTIOCHARES Banks
 Pronotum arcuate behind; propodeum densely hairy above
 LOPHOPOMPILUS Radoszowski
3. Propodeum not hairy above; small or rather small species
 POMPILOIDES Radoszowski
 Propodeum hairy above. 4
4. No spines under last joint of hind tarsi; third submarginal cell not petiolate
 nor triangular; abdomen with red in our species
 SERICOPOMPILUS Ashmead
 Last joint of hind tarsi with spines on under side. 5
5. First and third antennal joints subequal in female; pronotum almost transverse
 behind. SOPHROPOMPILUS Ashmead
 Third antennal joint plainly longer than first. 6

6. No comb on front tarsus of female
A comb on front tarsus of female

ANOPLIUS Lepeletier
PSAMMOCHARES Latreille

Subgenus NOTIOCHARES Banks

Clypeus emarginate in front, less so in males; pronotum angulate behind; propodeum only scantily hairy above. One large species belongs here.

- P. (N.) philadelphicus* Lep. Length, male 12-16, female 18-25. Wholly black including wings Found throughout the state from Buncombe County eastward, flying from May to October and probably the commonest of the Psammocharids.

Subgenus LOPHOPOMPILUS Radoszowski

Clypeus emarginate in front, much less so in males; hair on upper surface of propodeum dense and the pronotum arcuate behind.

Six forms have been reported from North Carolina.

KEY TO OUR SPECIES OF LOPHOPOMPILUS

- | | |
|---|-------------------------|
| 1. Wholly black species | 2 |
| Second abdominal segment largely orange above | 4 |
| 2. Emargination of clypeus in female narrow and clear cut; four long spines on front metatarsus of female | <i>cleora</i> Banks |
| Emargination of female clypeus much broader and less clear cut | 3 |
| 3. Emargination of female clypeus well developed; three spines on front metatarsus of female | <i>aethiops</i> Cresson |
| "Clypeus barely emarginate in front" (Banks) | .. <i>ilione</i> Banks |
| 4. Smaller, about 10-12; pleura with little or no hair | <i>carolina</i> Banks |
| Larger, 18-20; pleura with plentiful hair | 5 |
| 5. Lateral ocelli nearer to each other than to the eyes; nervulus slightly beyond basal vein, rarely interstitial | <i>atrox</i> Dahlbom |
| Lateral ocelli nearer the eyes than each other; nervulus interstitial or before basal vein | <i>bengtssoni</i> Regan |

- P. (L.) aethiops* Cr. Length 17-20. Third submarginal cell much narrowed above so that it is triangular or sub-triangular. Raleigh, Statesville, June to November.

- P. (L.) atrox* Dahl. Length 15-20. Throughout the state, April to November.

- P. (L.) bengtssoni* Regan. Size of *atrox* and differs as per key. Smith Island, October, F. Sherman. I have not seen it.

- P. (L.) carolina* Bks. The type locality is "Black Rock," N. C., apparently a slip for Black Mountain or Blowing Rock. We have two females from Highlands, September, and Boone, summer,

1934. Have also seen one taken by Dr. T. B. Mitchell at Cruso in June.

- P. (L.) cleora* Bks. Length 18. Elizabeth City, August; Raleigh, June, two females.
- P. (L.) ilione* Bks. Length 13-15. Described from Southern Pines, N. C. Regan makes it a synonym of *aethiops*, and Banks thinks the eastern specimens referred by Regan to *cleora* are this species. I have not seen it.

Subgenus POMPILOIDES Radoszowski

Rather small species without erect hair on the propodeum. As employed here the genus includes species placed by Banks in 1917 in *Nannopompilus* and *Arachnophila*.

KEY TO NORTH CAROLINA SPECIES OF POMPILOIDES

1. Pronotum margined behind with white; males only 2
Pronotum not margined with white 3
2. Abdomen partly red above *agnema* Brimley
Abdomen wholly black *albomarginatus* Banks
3. Abdomen partly red; females only 4
Abdomen wholly black; both sexes 8
4. Abdomen with red on first three tergites and on the corresponding sternites also 5
Abdomen with red on tergites only; females without tarsal comb 6
5. Pronotum arcuate behind; female with comb on front tarsus *pretiosa* Banks
Pronotum angulate behind; female without tarsal comb... *semirufus* Cresson
6. Third submarginal cell not usually petiolate; red on tergites 2 and 3 only *americanus* Beauvois
Third submarginal normally petiolate; segment 3 usually without red. ... 7
7. Segments 1 and 2 largely red above *marginatus* Say
Red confined to second segment only *reducta* Banks
8. Front tarsus of female with comb of long spines; first and third antennal joints of female subequal; third antennal joint of male short, shorter than fourth; pronotum arcuate behind *argenteus* Cresson
No comb on front tarsus of female; third joint of male antennae as long as or longer than fourth 9
9. Pronotum arcuate behind 10
Pronotum angulate behind 11
10. Third antennal joint of female subequal to first; propodeum without longitudinal furrow *minora* Banks
Third antennal of female longer than first; propodeum with a longitudinal furrow. *subcylindricus* Banks
11. Third submarginal cell receiving second recurrent well beyond its middle; propodeum with longitudinal furrow *insolens* Banks
Third submarginal receiving second recurrent at middle 12

12. Third submarginal petiolate; propodeum without longitudinal furrow

cylindricus Banks

Third submarginal not petiolate, the vein between it and second submarginal straight and perpendicular to its base; propodeum with longitudinal furrow

rectus Banks

- P. (P.) agnema* Brimley. Length 9. Pronotum angulate behind; body with much appressed silvery pubescence; wings hyaline, dusky at tip; red confined to two spots on second tergite; third submarginal petiolate. Two males, Raleigh, July, and September. These may be males of *reducta*. A third male taken at Raleigh, August 17, 1930, has red on part of tergites 1 and 3 as well as on 2, and on the corresponding sternites also; third submarginal subtriangular, not petiolate; this may be the male of *semirufus* but is placed here for the present.
- P. (P.) albomarginatus* Banks. Resembles preceding but the abdomen is wholly black. Length 6-10. Raleigh, Kingsboro, June to September, seven males. Third cubital subtriangular in one, petiolate in the rest. Cannot associate this with any particular species known in the other sex.
- P. (P.) americanus* Beauvois. Length 10-15. Wings dusky; third submarginal usually not petiolate; red on tergites 2 and 3, not on 1. Raleigh, Kingsboro, June to early October, rather common.
- P. (P.) argenteus* Cresson. Length 6-8. Wings hyaline tipped with dusky; body with much appressed silvery pubescence; third submarginal subtriangular to petiolate. Distinguished by small size, arcuate pronotum, and long spines on front tarsus of female. Beaufort, one male; Raleigh, two females, six males; May, July, September.
- P. (P.) cylindricus* Cresson. Length 8-10. Wings dusky in females, hyaline tipped with dusky in males. Raleigh, Spruce, Highlands, May, June, September.
- P. (P.) insolens* Banks. Length 9. Described from Black Mountain and we have a specimen from Hendersonville (June) determined by Banks.
- P. (P.) marginatus* Say. Length 10-12. Averages somewhat smaller than *americanus*; third submarginal almost always petiolate and red present on tergites 1 and 2, not on 3. Raleigh, Burgaw, Wendell, Wadesboro, Highlands, May, July to September.
- P. (P.) minora* Banks. Described from Southern Pines. I have not seen it.

- P. (P.) pretiosa* Banks. Length 8. Wings dusky; first three abdominal segments reddish above and below. Raleigh, May; Hendersonville, June, two females.
- P. (P.) rectus* Banks. Some of the type material was from Black Mountain. Two males from Raleigh, May and September.
- P. (P.) reducta* Banks. Similar to *marginatus* but red on tergite 2 only and may be only a variation of that species and not the true *reducta* of Banks. Raleigh, Wilmington, Kingsboro, Pembroke, Oriental, and Marion, June to October.
- P. (P.) semirufus* Cresson. Raleigh, June, September, four females.
- P. (P.) subcylindricus* Banks. Raleigh, June, July, October, three females.

Subgenus SERICOPOMPILUS Ashmead

Marginal cell comparatively long and narrow, third submarginal long, wide above, never triangular, subtriangular or petiolate; no spines under last joint of hind tarsi; claws toothed near base in both sexes. All our species have the abdomen marked with red.

KEY TO SPECIES OF SERICOPOMPILUS

1. Mid and hind tarsi with joints largely white; pronotum margined behind with white; males *cinctipes* Cresson
No white on the tarsi or pronotum; females 2
 2. Basal abdominal segments reddish above and below; legs black
fuscipennis Lepeletier
Abdomen wholly red, legs partly so *georgiana* Banks
- P. (S.) cinctipes* Cr. Length 10-11. Wings whitish hyaline broadly tipped with black; joints 1-4 of mid tarsi and 2-4 of hind tarsi white tipped with black; a white stripe at base of hind tibiae above; second abdominal segment mainly red. Raleigh, Southern Pines, Jonesboro, June. Probably the male of *fuscipennis*.
- P. (S.) fuscipennis* Lep. Length 15. Two basal abdominal segments red; wings nearly uniform black. Wilmington, Kittyhawk, July; Raleigh, June.
- P. (S.) georgiana* Banks. Abdomen wholly red; hind tibiae and tarsi usually red. Raleigh, August; Harkers Island, June; Carolina Beach, September; Southern Pines (type locality) June, August

Subgenus SOPHIROPOMPILUS Ashmead

Female with first and third antennal joints equal or the third a little the longer, and with comb on the front tarsus; pronotum usually trans-

verse behind; spines present beneath last joint of hind tarsi; third submarginal not triangular nor petiolate though much narrowed above; wings more or less dusky tipped with darker. Wholly black or bluish species of small size.

KEY TO OUR SPECIES OF SOPHROPOMPILUS

1. Pronotum angulate behind, blackish species *tebemi* n. sp.
Pronotum arcuate behind, bluish species. 2
2. Four long spines on first joint of front tarsus of female; length about 12
ingenuus Cresson
Three shorter spines on first joint of front tarsus of female; length about 10 or less *hyacinthinus* Cresson

P. (S.) hyacinthinus Cr. Raleigh, May, July, Aug.; Swannanoa, June.

P. (S.) ingenuus Cr. Raleigh, Aberdeen, Lumberton, Tarboro, Asheville, Swannanoa, Wendell, April, May, July to October.

P. (S.) tebemi n. sp. Length 11 mm. Black, the wings pale fuscous, the front pair broadly tipped with blackish; antennae short and thick, the third joint slightly but distinctly longer than either the first or fourth; pronotum feebly but distinctly angulate behind; propodeum with a deep groove down its dorsal surface; second and third submarginal cells subequal, the former slightly, the latter much narrowed above, each receiving a recurrent vein well beyond the middle, the second transverse cubital wanting in the right front wing; nervulus slightly beyond, nervellus slightly before the basal veins of their respective wings; marginal cell rather short and broad; only three long spines on the first joint of front tarsus; longest spur of hind tibia four-fifths its metatarsus; claws with a single tooth near base; body with very little hair.

Type, a single female taken at Smokemont, North Carolina, by Dr. T. B. Mitchell, June 30, 1934.

The short antennal joint and erect but scanty hair on propodeum place this species in *Sophropompilus*, while the posterior angulation of the pronotum distinguishes it from the other species of the subgenus.

Subgenus ANOPLIUS Lepeletier

Wholly black species without a comb on the front tarsus of the females and usually with dense hair on the subapical ventral segments of the males.

KEY TO NORTH CAROLINA ANOPLIUS

1. Third submarginal cell petiolate above. *tenebrosus* Cresson
Third submarginal not petiolate. 2

2. Wings nearly evenly blackish; length 10-15. *illinoensis* Robertson
Wings nearly hyaline except at tip; length about 10. 3
3. No longitudinal groove on propodeum. *virginiensis* Cresson
A median longitudinal groove on upper surface of propodeum. *ithaca* Banks
- P. (A.) *illinoensis* Robertson. Length 10-15. Third submarginal much narrowed above; males with much dense hair on underside of subapical ventral segments. Both sexes from Raleigh, Wendell, Sanford, Pembroke, and Wadesboro, May to October.
- P. (A.) *ithaca* Banks. Length 10-11. Much like *virginiensis* but wings slightly darker and there is a longitudinal groove on the upper surface of propodeum. Raleigh, April, May, three females.
- P. (A.) *tenebrosus* Cresson. Wholly black, wings dusky. Raleigh, October; Southern Pines, March; Swannanoa, September. Length 10-15.
- P. (A.) *virginiensis* Cresson. Length 8-10. No median longitudinal groove on propodeum. Raleigh, May, June; Moncure, October; Swannanoa, May.

Subgenus PSAMMOCHARES Latreille

Comprises species either all black or with some red on the abdomen. There is a comb of long spines on the front tarsus of the female, the third antennal joint of the female is longer than the first, there are spines beneath the last joint of the hind tarsus and erect hair on the propodeum.

KEY TO NORTH CAROLINA SPECIES OF PSAMMOCHARES

1. Abdomen partly red 2
Abdomen wholly black 5
2. Red on abdomen mostly confined to upper surface of second segment; vertex rather wide. *fabricii* Banks
Red more extensive. 3
3. Pronotum edged with white behind, both scutella gray on the sides
autumnalis Banks
No white on pronotum nor gray on scutella. 4
4. Vertex narrow, narrower than in *fabricii*; spines on front tarsus of female short
eurydice Banks
Vertex as wide as in *fabricii* *marginalis* Banks
5. Third submarginal cell triangular; length over 15; prothorax arcuate behind
relativus Fox
Third submarginal subtriangular or trapezoidal; length not over 15. 6
6. Pronotum arcuate behind; males with tarsal claws toothed 7
Pronotum angulate behind; tarsal claws of males cleft. *astur* Banks
7. Bluish; marginal cell rather short, acute at tip; third antennal joint of male shorter than fourth. *scelestus* Cresson
Black; marginal cell long, almost rounded at tip; third antennal joint of male longer than fourth *gracilicornis* Banks

- P. (P.) astur* Banks. Length 10-13. Wholly black with pronotum angulate behind and third submarginal cell but little narrowed above. Raleigh, Wilmington, Pembroke, Wadesboro, Moncure, Aberdeen, Sanford, April to October; Highlands, Linville, Grandfather Mt., Swannanoa, July to October.
- P. (P.) autumnalis* Banks. Length 12. Black with light gray pubescence on anterior and posterior edges of pronotum, and on sides of scutella and of upper surface of propodeum; first two and base of third abdominal segments red above and beneath. Three females Oriental, June; Wilmington, May; Raleigh, May.
- P. (P.) eurydice* Banks. Length 12-14. Vertex plainly narrower than in *tropicus*; first, second and most of third abdominal segments reddish orange above and below; spines of tarsal comb short; third submarginal triangular. Two females, Raleigh, September; Kinston, June.
- P. (P.) gracilicornis* Banks. Type locality, Southern Pines, May. I have not seen it.
- P. (P.) marginalis* Banks. Much like *fabricii* but smaller and with red on first three abdominal segments. Type locality Southern Pines, from which place we have a female taken in August, also a pair from Aberdeen, close by, in October, and a male from Raleigh, July.
- P. (P.) relativus* Fox. Wholly black with pronotum arcuate and third submarginal triangular. Four females from Raleigh, July, October; Fayetteville, June; and Zebulon, September.
- P. (P.) scelestus* Cresson. Length 13. A single male from Kittrell in July.
- P. (P.) fabricii* Bks. (= *tropicus* L., preoccupied) Length 15. Black with large orange spot occupying basal three-fourths of second abdominal segment. Raleigh, Southern Pines, Aberdeen, Norlina, Faison, Castle Hayne, Wilmington, and Carolina Beach, June to October.

ARACHNOPROCTONUS Ashmead

Ferruginous species with median longitudinal groove on pronotum and upper edge of clypeus not notched. The species of this genus and the next look very much like the social wasps of the genus *Polistes*. Only one species is known to occur in the state.

- A. ferrugineus* Say. Length 10-20, the females much the larger. Wholly ferruginous, the propodeum darker and the legs lighter. Rather common at Raleigh, June to October, also taken at Blantyre,

Marion, Greensboro, Salisbury, Southern Pines, Oriental, and Southport.

BATAZONUS Ashmead

Ferruginous species varied with black and yellow; upper edge of clypeus notched on each side; a median longitudinal groove on the propodeum. Two species have been recorded from the state.

KEY TO NORTH CAROLINA BATAZONUS

1. Propodeum with yellow markings at its apex; mesonotum and coxae also usually with yellow markings.....*navus* Cresson
- Propodeum, mesonotum, and coxae without yellow markings.*interruptus* Say

B. interruptus Say. Much like the next but with more black and less yellow. Raleigh, two males, June and July.

B. navus Cresson. Length 10-16. Ferruginous, the thorax, propodeum and legs more or less black and marked variably with yellow; abdomen mostly ferruginous, banded variably with yellow; no two specimens alike. Both sexes, from Raleigh, Merry Oaks, Durham, Kittrell, Elizabeth City, and Pembroke, May to October.

DEPARTMENT OF AGRICULTURE,
RALEIGH, N. C.

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**PROCEEDINGS OF THE THIRTY-FIFTH ANNUAL MEETING
OF THE NORTH CAROLINA ACADEMY OF SCIENCE**

DUKE UNIVERSITY, DURHAM, N. C., APRIL 24 AND 25, 1936

The thirty-fifth annual meeting of the North Carolina Academy of Science was held at Duke University, April 24 and 25, 1936. The meeting was called to order at 9:30 A.M. on April 24, by the president, W. L. Porter, after which the presentation of papers commenced and continued until 11:30 when the president announced the appointment of the following committees:

Auditing: T. E. Powell, P. M. Ginnings, D. B. Anderson;

Resolutions: B. W. Wells, H. E. Fulcher, L. A. Whitford;

Nominating: J. B. Derieux, Z. P. Metcalf, J. P. Givler.

At 1:00 P.M. the Academy took a recess for luncheon.

The reading of papers was resumed at 2:00 P.M. and continued until 3:45 when the Academy held its annual business meeting.

The minutes of the previous meeting were approved as published in the Journal of the Elisha Mitchell Scientific Society.

Reports from the various committees were presented.

The executive committee, consisting of W. L. Porter, the president of the Academy, F. W. Sherwood, vice-president, H. L. Blomquist, secretary and treasurer, E. T. Browne, W. E. Speas, and H. R. Totten reported as follows:

"The executive committee met in Durham on April 24 and on April 25 with all members present.

"The committee appointed E. T. Browne to act as temporary assistant to the secretary during the meeting.

"Favorable action was taken on the request by Dr. William deB. MacNider that his paper be transferred to Friday afternoon.

"Two papers which arrived too late to go on the printed program were added to the program.

"The secretary-treasurer was authorized to purchase an interval-timer for the Academy.

"A special request made by Dr. Archibald Henderson and Dr. W. W. Elliott that Dr. Kasner of Columbia University be asked to speak twenty-five minutes before the Mathematics Section was granted.

"The committee voted to change the time of the business meeting from 4:30 to 3:45 P.M.

"The committee reported as elected to membership since the last meeting the following:

- Mr. Harvey A. Bernhardt, Catawba College, Salisbury, N. C.
Miss Susan Blackwell, Catawba College, Salisbury, N. C.
Dr. Murray F. Buell, Dept. of Botany, N. C. State College, Raleigh, N. C.
Mr. Ed. H. Carter, Catawba College, Salisbury, N. C.
Mr. Joffre L. Coe, Box 652, Chapel Hill, N. C.
Mr. T. S. Coile, Dept. of Forestry, Duke Station, Durham, N. C.
Mr. James Doubles, Dept. of Botany, U. N. C., Chapel Hill, N. C.
Mr. Richard A. Edwards, Dept. of Geology, U. N. C., Chapel Hill, N. C.
Mr. John L. Etchells, Box 5323, Raleigh, N. C.
Miss Virginia Foil, Catawba College, Salisbury, N. C.
Dr. Coleen Fowler, Greensboro College, Greensboro, N. C.
Mr. M. E. Gardner, N. C. State College, Raleigh, N. C.
Dr. Joseph Greene, 331 Haywood Bldg., Asheville, N. C.
Mr. Clarence Harris, Route #2, Durham, N. C.
Dr. David H. Howard, Davidson College, Davidson, N. C.
Mr. V. A. Hoyle, Dept. of Mathematics, U. N. C., Chapel Hill, N. C.
Dr. Jack Levine, N. C. State College, Raleigh, N. C.
Dr. Alfred J. Maria, Box 4037, Duke Station, Durham, N. C.
Mr. Arthur C. Menius, Jr., Catawba College, Salisbury, N. C.
Miss Sarah M. Nooe, Queen's-Chicora College, Charlotte, N. C.
Mr. James R. Piland, N. C. State College, Raleigh, N. C.
Mr. Chilton E. Prouty, 1 Westwood, Chapel Hill, N. C.
Mr. George Rohde, Bausch & Lomb Optical Co., Washington, D. C.
Mr. Leland Shanor, Box 292, Chapel Hill, N. C.
Miss Rachel Smith, Catawba College, Salisbury, N. C.
Mr. T. E. Smith, Tobacco Exp. Station, Oxford, N. C.
Dr. Hertha Sponer, Dept. of Physics, Duke Station, Durham, N. C.
Dr. M. K. Veldhuis, Box 5042, Raleigh, N. C.
Dr. J. A. Wheeler, 416 Pittsboro St., Chapel Hill, N. C.
Mr. E. K. Whitner, Catawba College, Salisbury, N. C.
Mr. R. E. Wicker, Pinehurst, N. C.
Mr. H. C. Wilburn, Box 353, Waynesville, N. C.

"The following were reinstated to membership:

Miss Louise Adams, High Point College, High Point, N. C.
 Dr. David Carpenter, Dept. of Physics, Duke Station, Durham, N. C.
 Dr. F. W. Constant, Dept. of Physics, Duke Station, Durham, N. C.
 Mr. Harry T. Davis, N. C. State Museum, Raleigh, N. C.
 Dr. Archibald Henderson, Chapel Hill, N. C.
 Dr. S. W. Hoffman, Statesville, N. C.
 Prof. A. L. Hook, Elon College, N. C.
 Dr. Charles W. Hooker, Duke Medical School, Durham, N. C.
 Mr. W. E. Jordan, N. C. State College, Raleigh, N. C.
 Mr. F. B. Meacham, N. C. State College, Raleigh, N. C.
 Dr. J. C. Mouzon, Dept. of Physics, Duke Station, Durham, N. C.
 Dr. Walter Nielsen, Dept. of Physics, Duke Station, Durham, N. C.
 Dr. J. L. Stuckey, N. C. State College, Raleigh, N. C.
 Mr. L. L. Williams, 223 McCauley St., Chapel Hill, N. C.
 Dr. W. J. Wyatt, Wake Forest, N. C.

"The committee also reported the following losses during the year:

Lost by death:

Dr. T. G. Harbison,
 Dr. Horace W. Frink.

Lost by resignation because of removal from state:

Dr. Eugene P. Deatrick.

Lost addresses:

Mr. Norman B. Foster,
 Miss Dorothea R. McNutt,
 Dr. Louise Perry.

Dropped from the roll because of non-payment of dues:

Twenty-four former members.

The Treasurer's report was as follows:

Financial Statement of the N. C. A. S. April 23, 1936

Receipts

Balance on hand May 1, 1935:

Savings Account.....	\$476.59	
Checking Account.....	73.43	
	<hr/>	
	\$550.02	\$550.02

Dues:

1933.....	2.00
1934.....	2.00
1935.....	150.00
1936.....	206.00

Initiation fees:

1935.....	28.00
1936.....	42.00

430.00 430.00

Overpay on dues..... 6.00

Interest on savings..... 11.35

N. C. Section Am. Chem. Soc. share toward programs—1935
and 1936..... 10.00Total Receipts (\$26.00 transferred from savings to checking
account)..... \$1007.37 \$1007.37*Disbursements*

Stationery and printing..... 29.32

Programs—1936..... 28.50

Postage..... 1.77

Refund on overpaid dues..... 6.00

Books for H. S. Essay Prize..... 12.26

Clerical Assistance..... 67.00

Jour. of E. M. S. S.—1935. (\$200.00 from checking
and \$24.00 from savings account)..... 224.00

Charges on bank balance..... .50

Reprints of Proceedings and Constitution..... 15.60

Express on Proceedings..... .82

Express on H. S. Essays..... 1.61

Sec.-Treas. Commission..... 43.00

Refund Sec. Dues..... 2.00

Telegram..... .47

Receipt Book..... .52

Total Disbursements 433.37 433.37

Savings Account

May 1, 1935..... 476.59

Interest..... 11.35

487.94

Withdrawn, Nov. 22, 1935 (\$26.00 transferred to
checking account; \$24.00 to Journal)..... 50.00

Balance April 23, 1936..... 437.94

Total balance	574 00
In Savings Account	437 94
	136 06
Two dues on hand	4 00
Balance in checking account	132 06

The above report was made as of April 23, 1936.

Submitted by H. L. Blomquist, secretary-treasurer

Audited April 24, 1936 by

T. E. Powell,

P. M. Ginnings,

D. B. Anderson.

"The committee accepted the invitation of President Omwake of Catawba College to hold the thirty-sixth meeting in Salisbury.

"The executive committee made the following recommendations to the Academy:

1. That all bills presented in the treasurer's report be authorized and paid and that the report be printed when audited.

2. That Bert Cunningham be appointed to select the books to be presented to the winner of the High School Science Essay Prize, and that he be authorized to draw upon the treasury as much as \$25.00 for these books; and that the secretary be authorized to appoint a representative of the Academy to award the prize and draw upon the treasury for the payment of his expenses.

3. That the discussion of each paper be limited to five minutes.

4. That the secretary be instructed when asking for titles to papers to ask, where two or more papers are presented for the general program by the same person, that the member indicate which paper should be placed first; also that the secretary state that it may be necessary if the program is crowded to place the second paper on a supplementary list to be called for if the time permits, otherwise to be read by title only.

5. That Dr. J. S. Holmes, state forester, of the State Department of Conservation and Development, and Dr. I. H. Manning of the Medical School of the University of North Carolina be made life members. Both of these members joined the Academy in 1902 and have continued their memberships up to date.

6. That the abstracts for the proceedings be limited to 250 words.

7. That for 1936 the treasurer be authorized to pay to the Elisha

Mitchell Scientific Society \$300.00 instead of \$1.00 per member as was done in 1935.

8. That a committee of three be appointed to work out plans by which the Academy may assist in financing the Journal of the Elisha Mitchell Scientific Society."

The auditing committee reported that they had examined the accounts of the treasurer for the period May 1, 1935, to April 23, 1936, and found them correct.

The reports of the treasurer and auditing committee were accepted.

The committee on high school science, consisting of Bert Cunningham, chairman, H. B. Arbuckle, C. E. Preston, Mrs. B. W. Wells, Lena Bullard, and Nancy Eliason, reported as follows:

"The committee reports that it has carried on the usual activities, namely, aiding in the district and state meetings of the science divisions of the North Carolina Educational Association, and the conducting of the contest for the High School Essay Prize.

"Your committee believes that a general survey of the high school science situation should be made, more especially as to courses offered, laboratory time allotted, equipment and materials provided, and the general quality of the work offered.

"It also believes that there should be a closer federation of high school science teachers and that such a federation should have an annual meeting either at the time of the Academy meeting or the state meeting of the N. C. E. A., for the purpose of discussing problems and exchanging ideas.

"The committee selected as judges for the essay contest, R. N. Wilson (Duke), chairman, Mary E. Petty (W. C. U. N. C.), and W. A. Speas (Wake Forest). There were 22 essays entered in the contest. The judges reported that Carl Deal of the Boyden High School, Salisbury, N. C., won the prize with an essay entitled "The Sea, a Mine."

"The committee recommends the continuation of the contest, the fields during the coming year to be biology or any of its subdivisions and geography."

The report of the high school science committee was accepted and its recommendations approved. It was voted that the committee be continued.

The legislative committee, consisting of Z. P. Metcalf, chairman, W. L. Poteat, Wm. F. Prouty, had no report at this time, but was instructed to continue.

The report of the elective appraisal committee, consisting of P. M.

Ginnings, chairman, E. H. Hall, R. E. Coker, Mary Conrad Cleaver, C. W. Edwards, Karl Fussler, E. G. Purdom, R. N. Wilson, J. T. Dobbins, was as follows:

"At the request of Catawba College, a sub-committee made a visit to this institution and as a result made the following recommendations:

"1. That the administration of Catawba College be informed of the constructive criticisms of the appraisal committee.

"2. That the departments in the sciences of Catawba College be approved for a period of three years subject to the absence of any radical changes."

The above report was accepted and the recommendations were approved by the Academy.

The conservation committee, consisting of Charles E. Raynal, chairman, B. W. Wells, J. P. Givler, C. F. Korstian, W. C. Coker, and J. S. Holmes, did not have a report but recommended that the committee be continued.

The recommendation of the conservation committee was approved.

The committee on public school curriculum, consisting of C. E. Preston, chairman, Mrs. B. W. Wells, and Lena Bullard, did not present a report.

A motion made by the chairman that the committee be discharged was passed.

The provisional committee on the A. A. A. S. grant, consisting of R. E. Coker, chairman, Milton L. Braun, C. F. Korstian, R. F. Poole, William L. Poteat, made the following report:

"The committee on awards appointed by the president early last winter was instructed (1) to nominate a candidate for the A. A. A. S. grant for the current year, and (2) to propose to the Academy a policy to be followed with reference to this award which is understood to be an annual grant.

"Your committee fulfilled the first function by recommending to the president and executive committee that the award for the current year be granted to Dr. Karl Z. Morgan of Lenoir-Rhyne College for research on 'Scattering of Cosmic Rays'. The decision was arrived at after very careful consideration of a number of applications received in response to a circular letter sent out from the office of the secretary of the Academy.

"In regard to the matter of future policy, the present committee recommends the election of a permanent committee of five, the first committee to be composed of two members elected for a term of one

year, two for a term of two years and one for a term of three years; thereafter, vacancies to be filled as terms of members expire (two members one year, two the next and one every third year) by the election of members to serve for a term of three years.

"The committee recommends that the regular nominating committee of the Academy shall at each annual meeting of the Academy submit nominations for membership on the permanent committee on awards.

"The present committee offers two suggestions:

"(1) That the selection of members of the committee be governed as far as is practicable by the principle of rotation among institutions and fields of research in science.

"(2) That the committee on awards give a reasonable degree of consideration to the relative needs of applicants."

This report of the committee on the A. A. A. S. grant was accepted and the recommendations were approved.

The following memorial reports honoring the late Dr. T. G. Harbison and Dr. Horace W. Frink were presented:

DR. THOMAS GRANT HARBISON

Thomas Grant Harbison died at Chapel Hill, N. C., on January 12, 1936. He was born in Forest Hills, Union County, Pennsylvania, the son of Thomas V. Harbison, April 23, 1862. He early showed remarkable mental ability and began teaching at the age of seventeen. He attended school during vacations, and being near Bucknell University he continued his studies under professors of that institution even while teaching. He never had a continuous year as a resident college student, work in vacations, the work under professors of Bucknell University, and later a short course in the University of Norway and one in the University of Leipsic completing that type of his training; but he early began to build up his own library, and by the time he was twenty-one he had a library of over a thousand volumes. This library he continued to add to throughout his life, and aided by a keen power of observation and a very retentive memory he truly educated himself. The education that he built was not a narrow one, and did not consist of mere reading at random. He applied himself to definite courses of study as laid out by the best correspondence schools of the time. In this way he followed through a four year course of study as outlined by the University of the City of New York, several years' study in the National University, the botanical part here under Dr. Coulter of the University of Chicago. By these methods he received through correspondence

courses the B.S., the A.M., and Ph.D. degrees and completed a course in Landscape Architecture and Gardening.

In 1886 young Harbison and a close friend, E. E. Magee, spent the late spring and the summer in an extended walking trip down into the south. This trip took them across Maryland and eastern Virginia to Norfolk, across North Carolina to Asheville, down to Highlands, N. C., near the Georgia line, back across North Carolina, up through the Shenandoah Valley of Virginia, across Maryland and home. From Caesar these two young men had learned that troops can march and fight on a ration of grain. They took as their equipment on this trip, in addition to their clothing, a small water proof bag to carry their ground wheat and some brown sugar, a tin bucket for a stove, and each had a copy of Wood's *Botany*. Except for the fruits along the way, they had practically no other food than the wheat mush sweetened with brown sugar, and yet they returned in fine condition. On one day, towards the end of the trip, they walked fifty-two miles. This trip had a great influence in Dr. Harbison's future life. It proved to him his own rugged constitution, and he never had fear of hard work, long hikes, and mountains; his first hand knowledge of and love for plants were increased; and he became convinced that in our own North Carolina mountains is a most satisfying place for the abode of a lover of nature. He had great love and respect for his native part of Pennsylvania, and through correspondence with relatives and friends and through a county paper¹ he kept in touch with it to the day of his death. However, this trip was the direct cause of his becoming a North Carolinian.

On his return to his home in Pennsylvania, he found that the people of Highlands had sent a call for him to teach their school. He returned to Highlands in the fall of 1886, and though away at brief intervals, he maintained a home there until death, practically 50 years later. He was the principal in public and private schools there from 1886 to 1893 and from 1895 to 1896. He developed there unique and effective methods of instruction. Naturally, plants had a great part in it. Besides reading, writing and arithmetic, the usual things learned in elementary schools, his pupils learned plants, not only their common names, but their Latin names as well, and what the Latin names meant and while doing so built a foundation for a knowledge of English. Through very small prizes, a penny for the first flower of each kind for.

¹ The Lewisburg Saturday News. From the columns of this paper we have been able to secure a part of the information about him given in this article.

the season, five cents for the rare finds, he and they learned the flora of their region. And when funds ran low they kept their school going largely through shipping plants to northern markets. He became an advocate of an educational system that would provide a garden and work shop as a part of the elementary school.

The year 1893-94 Harbison traveled in Europe, studying the methods and results of such schools in Norway, Sweden, Denmark, Germany, and Switzerland. On his return he spent the following year lecturing on European schools and farming. For a time he edited a paper at Highlands. In 1896 he became principal of the Waynesville High School. One of the teachers in the school was his life-long friend and companion of his walking trip, E. E. Magee. The Waynesville High School was a private institution; but he set to work to have it taken over as a public school, and largely through his efforts it became one of the first public graded schools in the state. Also in 1896 he married Miss Jessamine M. Cobb, a daughter of Judd M. Cobb, the first building paper manufacturer in America, and a descendant of John Cobb, who came to Massachusetts in 1624 and built and operated the first iron foundry in America.²

The following year, 1897, he became a collector for the newly established Biltmore Herbarium of the estate of George W. Vanderbilt. The spring and early summer of 1898 he spent in studying and making collections of the plants in the eastern part of our state; the late summer and fall he spent in similar work in the Rockies, the Cascades, and the western Coast Ranges. He continued in such work, studying and collecting plants, mostly in our southern states, until the discontinuance of the herbarium at Biltmore in 1903. From 1905 to 1926 he was the southern representative for Dr. C. S. Sargent and the Arnold Arboretum, continuing the studying and collecting of southern flora especially the woody plants.

In 1929 he carried on botanical work for the Geological Survey of Mississippi. In 1933-34 his services were secured by the University of North Carolina to assist in assembling the Ashe Herbarium; and in 1934, at the age of seventy-two, at an age long after men of weaker build would have considered their work finished and well done, he was appointed Curator of the University of North Carolina Herbarium, the position he held at the time of his death. The University of North Carolina was most fortunate in securing his services for assembling

² In their Highlands home the Harbisons have the first iron crane made in America.

the Ashe Herbarium and directing its herbarium work. These two men, W. W. Ashe and T. G. Harbison, long friends and constant correspondents about the plants that they found in our southeastern states, and both prolific discoverers of new species, kept each other well informed of even the location of type plants from which the new species were described. These two, each with about a third of a century of field work, knew the woody plants of these southeastern states as no one else knew them. In his last illness, Mr. Ashe wrote Mr. Harbison, asking him in case of his death, to assist Mrs. Ashe in disposing of the Ashe Herbarium, and also expressed the hope that it could be secured by the University of North Carolina. It must be a considerable satisfaction to botanists everywhere that Mr. Harbison's own collection at Highlands has also since his death been secured by the University of North Carolina, and that the private collections of both of these men are to be found together in the same Herbarium, where they will continue to assist the present and later botanists in a way comparable to the assistance their collectors so freely gave to others while in life.

T. G. Harbison published the following botanical articles:

"New or Little Known Species of Trillium." *Biltmore Botanical Studies* 1: No. 1: 19. 1901, *Biltmore Botanical Studies* 1: No. 2: 158. 1902.

"A Sketch of Sand Mountain Flora." *Biltmore Botanical Studies* 1, No. 2. 151. 1902.

"Notes on the Genus Hydrangea." *American Midland Naturalist* 11: 255. 1928.

"Polycodium Ashei Harbisoni." *Midland Naturalist* 22: 179. 1930.

"Symlocos tinctoria Ashei, A New Dyebush from the Southern Mountains." *Journal of the Elisha Mitchell Scientific Society* 46: 218. 1931.

A Preliminary Check-List of the Ligneous Flora of the Highlands Region, North Carolina. *Highlands Museum and Biological Laboratory Publication* No. 3. 1931.

He had in manuscript a "Check List of the Woody Plants of Mississippi," results of field studies since 1915; also a "Check List of the Ligneous Flora of the Southern Appalachians," results of field studies since 1886.

The above list of publications hardly begins to measure the contributions of T. G. Harbison to the science of Botany. As a plant collector he recognized many new species and varieties of plants that he passed on to others for publication and he contributed greatly to the value of many publications through the gifts and loans of specimens and through his accurate memory as to the distribution of plants and the location of the types. His own herbarium is rich in type and co-type sheets.

He was very proud of the fact that he had visited nearly every type tree still living in the southeastern states, and the exact location of every type plant that he had ever seen seems to have been stamped indelibly upon his memory. We have been guided by him unerringly and with hardly any hesitation to type trees that he had not seen for thirty years. In recognition of his contributions to science the University of North Carolina chapter of the Sigma Xi elected him to active membership in 1935.

It is difficult to sum up briefly the life and contributions of this remarkable man. His work as a teacher in the schools of Pennsylvania and in the mountains of North Carolina was outstandingly successful and to the day of his death he was known and loved in our mountains as "Professor Harbison." His advocacy and influence helped to establish at Waynesville one of the first public graded schools in the state. In the same quiet way he was quite an influence in the establishment of the Western Carolina State Teacher's College at Cullowhee, N. C. He was an early advocate of manual training as part of the curriculum in each elementary school. As an active, influential member of the Junior Order and the Order of Masons he furthered the cause of education through those organizations. In his later years as a nature instructor in summer camps he continued the type of educational work that was nearest his heart. His public spirit was boundless. For a time he was mayor of his town; was for years road supervisor of his township and ever took pride in his part in the placement of some of the mountain roads. He was a pioneer in securing national forests for the western part of North Carolina, turned over much of his own land to the government at a very low rate and by his advocacy and example persuaded his neighbors to do the same. It should be mentioned here, however, that his last appearance before the North Carolina Academy of Science was in protest against a short-sighted National Forest policy of considering the purpose of National Forests almost solely that of raising timber trees and these of only a few kinds. Though a lover of timber trees, he also knew and loved all humbler plants too and fully realized their cultural value. He was an early advocate for a great National Park in the North Carolina mountains and was an early contributor and played a part in securing the cooperation of the mountain people in what has resulted in the Smoky Mountain National Park. He supervised the laying out and planting of a number of estates and through his knowledge of plants and their requirements, and through an innate sense of fitness and beauty he would open up distant vistas

and at the same time preserve the natural beauty of the native vegetation. As an orchardist he found and taught by experiment what varieties would be most successful in that apple region where North Carolina and South Carolina and Georgia meet. On his orchard farm on the southern slope of Mount Satulah he also conducted mountain tests as to the hardiness of new crops tried out or developed by the South Carolina Agricultural College and Experimental Station at Clemson, S. C. For years his family has kept the government weather records as to temperature and rainfall at the Highlands, N. C., station.

Harbison had a very influential part in the establishment and maintenance of the Highlands Library, the Highlands Museum, the Highlands Biological Laboratory. For years he gave freely his services as guide to practically every botanist and even to those only mildly interested in botany coming into the Highlands section. Up to within a year of his death when he was forced to give up strenuous mountain climbing by a severe attack of influenza that left his heart weakened, he could set a pace up mountain trails that any man half his age would have been proud to maintain. We have been on hard fifteen mile mountain hikes with him when he was past seventy years old. The memory of these mountain hikes and long rides, the botanical excursions through the mountains, the piedmont, the sandhills and coastal region of North Carolina, South Carolina, Georgia and Florida, the example of his keen and accurate observation, long talks at evening after the day in the field or herbarium (a memory and active life such as his made him a wonderful conversationalist), the patient help he gave to students makes us realize that in the passing of this cultured gentleman, scientist, we have lost a great teacher and friend. It was quite fitting that Thomas Grant Harbison should have spent his last days in the service of his state, in an honored position in its university. We wish that he could have carried on for years more the very valuable work he was doing and for which he was so well equipped. However, the thought of the loss is far outweighed by the thought of nearly seventy-four years of life as he lived it. While we send our sympathy to his wife, his three daughters, his son and his brother, still more we rejoice with them that Thomas Grant Harbison has lived.

H. R. TOTTEN,
W. C. COKER,
H. J. OOSTING, •
Committee.

DR. HORACE WESTLAKE FRINK

Horace Westlake Frink was born at Iroindale, N. Y., February 7, 1883. He died at a sanatorium near Southern Pines, N. C., April 18, 1936. His medical education was received at Cornell University Medical College, where he received the degree of Doctor of Medicine in 1905. Though first specializing as a surgeon, he early became interested in nervous and mental diseases, their psychological manifestations and therapy. For a time he studied under Sigmund Freud in Vienna, and he devoted the remainder of his life to work in these fields and attained eminence in them.

From 1914 to 1922 he was assistant-professor of neurology in Cornell University Medical College and was assistant attending neurologist at Bellevue Hospital, New York City. In 1922 he retired from teaching to devote himself to research and writing in psychology and psychiatry. He was the author of *Morbid Fears and Compulsions; Their Psychology and Psychoanalytic Treatment, Dreams and Neuroses*, and other titles. For a time he was associate editor of *The International Journal of Psychoanalysis*.

Among the learned societies to which he belonged are: the New York Neurological Society, the New York Academy of Medicine, the Neurological Association, the New York Psychoanalytic Society, of which he was president for three years, and the Psychopathic Association, of which he was secretary in 1915.

Having found in this region a location favorable to his not very vigorous health and congenial to his interests, he moved to Chapel Hill in 1932 where he subsequently bought a house and established his home. Coming to Chapel Hill without any connection with the University, he soon acquired a group of friends who found in him a man of worth and accomplishment and sincere genial manners. Though distinguished for modesty rather than assertiveness, his varied interests and talents brought him into contact with many phases of community and university life. Athletics and aesthetic crafts as well as scholarly and professional matters were subjects of eager interest to him. Though not wishing to build up a practice as a physician, his extensive knowledge and special talents in the field of psychiatry, coupled with his sincere desire to help others where he could, soon brought him into the relationship of friend and helpful adviser to many of those afflicted with or having to deal with nervous disorders. Though having no official connection with the University, he very generously and ca-

pably served as occasional special lecturer to the classes in neuro-anatomy of the Medical School and to the students in the Department of Psychology.

It was typical of his interests and coöperative nature that shortly after coming to the state Dr. Frink affiliated himself with the North Carolina Academy of Science and took an active interest in both the social and scientific aspects of its sessions. In his death the Academy has suffered the loss of a valued and distinguished member. To his son and daughter, his widow, and other members of his family, the Academy wishes to extend its deepest sympathy in their grief.

J. B. BULLITT,
I. H. MANNING,
W. C. GEORGE,
Committee.

The general resolutions committee, consisting of B. W. Wells, chairman, H. E. Fulcher, and L. A. Whitford, reported as follows:

"It is with great regret that we record the recent deaths of Dr. Horace W. Frink, Dr. Julia Dale, and Dr. Charles F. Meserve, former president of Shaw University.

"Dr. Frink was a psychoanalyst formerly on the faculty of Cornell University. He resided in Chapel Hill. He died at the age of 53.

"Dr. Julia Dale, for several years assistant professor of mathematics at Duke University, was at one time an active member of the Academy.

"President Meserve was for many years an active member of the Academy. Though his major field of activity was not that of science, he was profoundly interested in biological science and geology. In his later years he was an active member of the Raleigh Natural History Club. He died at the age of 86.

"Through your resolutions committee the North Carolina Academy of Science desires to express its great appreciation of the manner in which Duke University has entertained North Carolina's leading science organization. The opportunities to visit the great Duke Hospital and the extensive Duke Forest proved to be especially instructive experiences. The tea, dinner, and organ recital, so generously given to the Academy contributed to the social side of the meeting and were much appreciated. We give voice to the feeling of every member attending when we state that the Academy is deeply grateful for all the courtesies shown by its host, the faculty of Duke University, under the chairmanship of Dr. Bert Cunningham."

Bert Cunningham, representative of the Academy to the A. A. A. S., reported as follows:

"The Academy Conference for 1935-36 was largely devoted to the problem of Junior Academies, with some injection of the problems of branches of the A. A. A. S.

"The session was opened with a History of the Accomplishments of the Academy Conference by H. E. Enders. Secretary Bilsing rapidly sketched some of the things which the Academy Conference might do. In a later conference your representative found that other academies were not much interested in the publication of Academy programs in Science.

"Watson Davis pointed out that a publication was desirable for the Junior Academies and suggested devoting a portion of the Science News Letter to this service. A rather extended report was made on coördination of science clubs, and source material for them.

"The increase in high school science clubs has made it desirable to have them affiliated with each other in order to make possible a more satisfactory method of securing and distributing source materials, programs, and working ideas. Since the move is supposedly scientific it seemed desirable to have the various groups under the eye of some scientific organization. The State Academy seemed to be the most available and the most satisfactory foster parent—especially if some one in the Academy would assume the directorship of such an organization.

"A conference with Dr. Caldwell, chief proponent for the clubs, led to clarification of the situation, both from his standpoint and from ours. It was pointed out to Dr. Caldwell, and he concurred, that the high school committee of our Academy in its present functioning was probably serving the function of director; that the loose association which the Academy maintains with science teachers in the secondary schools is satisfactory in a diffuse population such as occurs in North Carolina; that a federation of clubs might for the same reason be undesirable and that for the "federated" clubs to meet at the time and place of the Academy would be impractical.

"In conferences with Dr. Ward we were commended upon our high school prize plan, and were asked by him to submit details of our plan. It was made clear by both that no pressure would be brought to bear for a Junior Academy in this state.

"Your representative concurs with the Academy conference that a publication devoted to high school science and high school science clubs would be highly desirable, but doubts the advisability of diverting a part of the Science News Letter to that end.

"The report on research grants indicated that many of the academies accepted and approved of the change. It was quite apparent from the general secretary's financial report to the A. A. A. S. that the change was imperative. The funds for current expenses (from which the Academy rebates were made) have for several years fallen short of expenditures. On the other hand the Association has a fund which can be expended only for research, and it is from this fund that the grants are now made. Assurance was given that similar grants would be made for the coming year. Your representative agrees that the present procedure is justified under the circumstances.

"The problem of local branches of the A. A. A. S. was mentioned here as well as in the Council meetings. To your representative it seems that a local branch would in no way interfere with the Academy. The branch has a more or less popular meeting and is not so much interested in new contributions, as such, as in the stimulation of scientific interest and the dissemination of scientific knowledge. Membership is not restricted to members of the A. A. A. S., and the move is not to increase membership in that organization. It is desirable from the standpoint of the A. A. A. S. that the Academy sponsor and coöperate with local branches.

"The time remaining for the discussion of other problems was insufficient and informal discussion was carried on at the dinner.

"It seems to your representative that little of the primary purpose of the Academy Conference has been achieved during the several years of its existence, and it is to be hoped, that Junior Academies and A. A. A. S. branches will give way to more thoughtful discussion of how the academies may improve themselves by profiting by the experience of others.

"While for the most part the Council meetings were devoted to routine affairs there were some items of especial interest to this Academy. Two of these are dealt with in the report on the Academy Conference. Another was the establishment of a Southeastern Division of the A. A. A. S. Your representative pointed out that North Carolina being a "border state," would most likely prefer to meet with the A. A. A. S. if its meetings were within range, and that a meeting in the Southeast will probably be feasible, so far as this state is concerned, only when the general meeting is far distant.

"The Georgia Academy invited representatives of the various academies of the Southeast to meet with them at the time of their annual meeting and discuss the problem. Representatives of the A. A. A. S. were also invited. Although the meeting has been held your representative does not know what action, if any, was taken.

"It appears to your representative that it would be more feasible to have a loose federation of Academies of the Southeast which could have a Christmas meeting when the A. A. A. S. meets at some distant point. Such meeting should be centrally located and planned for several years in advance. Since the next two meetings are to be held at Atlantic City and Richmond, it is doubtful if any meeting should be held within the next two years."

The nominating committee submitted the following nominations:

President: P. M. Ginnings, Greensboro College;

Vice-President: C. F. Korstian, Duke University;

Secretary-Treasurer (for three years): H. L. Blomquist, Duke University;

New Member of the Executive Committee (for three years): W. L. Porter, Davidson College.

Committee on A. A. A. S. grant:

R. E. Coker (3 years), chairman,

M. L. Braun (2 years),

O. J. Thies (2 years),

J. G. Boomhour (1 year),

Eva G. Campbell (1 year).

The nominations were accepted and the secretary was instructed to cast the ballot of the Academy for the nominees.

The president then announced the appointment of the committee to assist the Elisha Mitchell Scientific Society to finance the Journal, as follows: Z. P. Metcalf, H. R. Totten, and H. L. Blomquist.

The business meeting then adjourned.

At 8:00 P.M. the members of the Academy were entertained at a complimentary dinner given by Duke University in the Union reception room.

At the conclusion of the dinner, with F. W. Sherwood, vice-president presiding, an address of welcome was made by W. C. Davison, Dean of the Duke University Medical School. This was followed by the presidential address, entitled "The Teacher of Science" by the retiring president, W. L. Porter.

On Saturday morning the Academy met in sections. President Porter presided over the General Section; W. N. Mebane, Jr., over the Mathematics Section; C. C. Hatley, over Physics Section; and R. W. Bost, over the Chemistry Section.

The following officers were elected by the respective sections:

Mathematics Section—Chairman, J. H. Roberts, Duke University;
Secretary, J. M. Clarkson, North Carolina State College.

Physics Section—Chairman, M. L. Braun, Catawba College; Secretary, J. S. Meares, North Carolina State College.

North Carolina Section of the American Chemical Society—Chairman, Edward Mack, Jr., The University of North Carolina; Vice-chairman, W. C. Vosburgh, Duke University; Secretary-treasurer, E. C. Markham, The University of North Carolina; Councilor, L. A. Bigelow, Duke University; Executive Committee, the officers, R. W. Bost, The University of North Carolina (3 years): C. S. Black, Wake Forest College (2 years); W. A. Reid, North Carolina State College (1 year).

The following papers were presented. Those marked with * appear in full in this issue. Those marked x are abstracted with the Proceedings. Those marked † were read by title.

GENERAL SECTION

**The Effects of Increased Atmospheric Pressure on the Developmental Rate of Chick Embryos* (Final Report) (Lantern). BERT CUNNINGHAM, Duke.

x*Faunal Statistics of North Carolina*. C. S. BRIMLEY, N. C. Dept. Agr.

x*The Effect of Periodic Illumination upon Cell Wall Structure* (Lantern). DONALD B. ANDERSON, State.

Stimulation of Seedling Plants by Organic Matter (Lantern). J. R. PILAND and L. G. WILLIS, N. C. Agr. Exp. Sta.

x*Treatment of Coastal Plain Waters for Domestic and for Industrial Users*. E. E. RANDOLPH, State.

Some of the Rarer Orchids of North Carolina. DONOVAN S. CORRELL, Duke.

xA *New Phosphorescent Species of the Genus Achromobacter*. FRANK H. JOHNSON and IVAN V. SHUNK, State.

New and Little Known Algae from North Carolina. L. A. WHITFORD, State.

Paper appeared in full in this Journal 52: 93. 1936.

x*Morphology and Hormone Production by the Testis in Relation to Age* (Lantern). C. W. HOOKER, Duke.

The Vegetation of a "bare-faced" Cliff in Western North Carolina (Lantern). HENRY J. OOSTING and LEWIS E. ANDERSON, Duke.

Physiological Studies at High Altitudes (Lantern). F. G. HALL, Duke.

An Electro-mechanical Device for Determining Humidity under Pressure (Demonstration in Room 223). WILLIAM HURST and BERT CUNNINGHAM, Duke.

The Effect of Sodium, Potassium, and Calcium Ions on Permeability of Amoeba proteus to water. COLEEN FOWLER, Greensboro College.

- xChanges in the Vegetation of the Forest Floor following Elimination of Root Competition* (Lantern). C. F. KORSTIAN, Duke.
- †*Thermal Zones in the Carolina Mountains*. MARTHA E. NORBURN, Biltmore.
- xInactivation of the Virus of Common Tobacco Mosaic in Soil* (Lantern). S. G. LEHMAN, State.
- The Acquired Resistance of Fixed Tissue Cells* (Lantern). WILLIAM DEB. MACNIDER, U. N. C.
- Resistance of the Air to (a) a Base Ball, (b) a Tennis Ball* (Opaque Lantern). J. B. DERIEUX, State.
- Carolina Bays and Current Explanations* (Opaque Lantern). WILLIAM F. PROUTY, U. N. C.
- xThe Osmotic and Porous Properties of Oil Films*. CHARLES M. HECK, State.
- Old Age Characteristics of the Ostracod Genus Ctenobolbina* (Opaque Lantern). RICHARD A. EDWARDS, U. N. C.
- xThe Susceptibility of Stretched Rubber to Temperature Changes*. MILTON L. BRAUN, Catawba.
- The Paleontologist's View of Species*. J. W. HUDDLE, U. N. C.
- xThe Relation between Rate of Transpiration and Rate of Water Absorption in Plants* (Lantern). PAUL J. KRAMER, Duke.
- Origin of Southern Appalachian Grass Balds* (Lantern). B. W. WELLS, State.
- xAuto-narcosis in the Oyster and its Industrial Application*. HERBERT F. PRYTHERCH, U. S. Fisheries, Beaufort.
- xComposition of the Leaf Litter of Forest Trees* (Lantern). T. S. COILE, Duke.
- The Soil Factor in Crop Adjustment*. J. F. LUTZ, State.
- Use of Trisomics in Linkage Studies*. H. S. PERRY, Duke.
- xThe Embryo Sac of Acorus Calamus L.* (Lantern). MURRAY F. BUELL, State.
- Peat Mosses (Sphagna) of North Carolina*. H. L. BLOMQUIST, Duke.
- Chromosomes in the Germ Cells of Epilachna corrupta Mulsant*. H. M. PHILLIPS and O. C. BRADBURY, Wake Forest.
- Production of mature Perithecia of Cordyceps militaris Link in Culture*. LELAND SHANOR, U. N. C.
- Paper appeared in full in this Journal 52: 99. 1936.
- Water Absorption by Reptile Eggs during Incubation* (Lantern). BERT CUNNINGHAM and A. P. HURWITZ, Duke.
- The Effects of Endocrine Feeding of Flesh-Fly Larvae* (Lantern). E. C. HESTER and BERT CUNNINGHAM, Duke.

The Tracing of Basic Dikes by Electrical and Magnetic Geophysical Methods. W. R. JOHNSON, JR., G. R. MACCARTHY, J. C. MACCAMPBELL, and H. W. STRALEY III, U. N. C.

Some Ostracods from the Sneedville Limestone of Eastern Tennessee. HOWARD E. VITZ, U. N. C.

MATHEMATICS SECTION

x*Geometrical Transformations.* EDWARD KASNER, Columbia University.

x*Some Remarks on Stieltjes Integrals.* F. G. DRESSEL, Duke.

x*Some Theorems on Tensor Differential Invariants.* JACK LEVINE, State.

x*On the Equation of Conics of r -point Contact.* J. W. LASLEY, JR., U. N. C.

Equilibrium Point of Green's Function for a Spherical Shell. A. J. MARIA, Duke.

x*Algebraic Systems.* J. M. THOMAS, Duke.

PHYSICS SECTION

x*The Effects of Acids and Bases upon the Infra-red Absorption Bands of Water (Lantern).* E. K. PLYLER and E. S. BARR, U. N. C.

x*Heat Transfer across an Interface between Oil and Water.* C. M. HECK and GRADY W. BARTLETT, State.

Resistance of the Air to (a) a Basket Ball, (b) a Foot Ball, sidewise and endwise (Opaque Lantern). J. B. DERIEUX, State.

Radioactive Fluctuations. A. E. RUARK, U. N. C.

Infra-red Absorption of HCl in Benzene. DUDLEY WILLIAMS and E. K. PLYLER, U. N. C.

On Photochemical Processes in Solutions. H. SPONER, Duke.

An Attempt to Obtain a Proton Source by Diffusion through Palladium. S. T. MARTIN and F. W. CONSTANT, Duke.

Infra-red Absorption of Phenol and Alcohol. J. W. WHITE and E. K. PLYLER, U. N. C.

Fine Structure in Nuclear Disintegrations. J. A. WHEELER, U. N. C.

The Design and Control of a High Current Mercury Arc. R. T. DICKERSON and W. M. NIELSEN, Duke.

Investigations on the Operation of a Van de Graaff Generator. C. H. TOWNES and F. W. CONSTANT, Duke.

Shower Production in Lead and Other Elements. J. E. MORGAN and W. M. NIELSEN, Duke.

The Absorption of Cosmic Ray Shower Particles in Lead and Other Elements. J. E. MORGAN and W. M. NIELSEN, Duke.

A New Voltage Regulator Circuit. J. A. ASHWORTH and J. C. MOUZON, Duke.

A Simple Method of Discriminating and Measuring Multiple Coincidences in Geiger Müller Counters. J. C. MOUZON, Duke.

EXHIBITS

Luminous Bacteria of the Genus Achromobacter. FRANK H. JOHNSON and IVAN V. SHUNK, State.

Morphology and Hormone Production by the Testis in Relation to Age. C. W. HOOKER, Duke.

Vegetation of North Carolina in Photographs. H. L. BLOMQUIST, Duke.

Peat Mosses of North Carolina. H. L. BLOMQUIST, Duke.

NORTH CAROLINA SECTION OF THE AMERICAN CHEMICAL SOCIETY

The Zinc Electrode. WILLIAM C. CLAYTON and WARREN C. VOSBURGH, Duke.

The Determination of Small Amounts of Alcohol and Water in U. S. P. Ether. F. H. SMITH, State.

Aldoximes and their Acyl Derivatives. C. R. HAUSER and E. JORDAN, Duke.

Double Sulfates containing Ferric Sulfate. FRANK K. CAMERON, U. N. C.

Partial Pressure of Hydrogen Chloride from Benzine Solutions. J. H. SAYLOR, Duke.

Business Meeting.

Oils and Waxes in Whole Cotton Plant. A. R. MACORMAC and DAVID MILNE, U. N. C.

The Accuracy of the Vitamin B. Assay. F. W. SHERWOOD, State.

The following abstracts have been received:

Faunal Statistics of North Carolina. C. S. BRIMLEY.

Summarises the fauna of the state as represented on the cards of the Division of Entomology of the State Department of Agriculture as follows:—

Total forms recorded, 11,560; of which 977 are vertebrates and 10,583 invertebrates.

The vertebrates include 90 mammals, 370 birds, 72 reptiles (including 1 crocodilian, 42 snakes, 9 lizards, and 20 turtles), 65 amphibians (of which 27 are frogs and 38 salamanders), and 380 fishes.

The invertebrates include 8 protochordates, 9,754 arthropods (of which 9,057 are insects, 368 arachnids, 265 crustaceans, and 64 myri-

apods), 197 worms, of all groups, 378 mollusks, 24 echinoderms, 88 coelenterates and sponges, and 134 protozoans.

The insect total of 9,057 is divided among 23 orders, of which the most important are Coleoptera with 2,919, Diptera, 1,638, Hymenoptera, 1,502, Lepidoptera, 1,070, Homoptera, 590, Hemiptera, 497, Orthoptera, 220, Odonata, 126, Mallophaga 119, the other 14 orders containing less than a hundred species each on record. The smallest order however, the Isoptera, containing the termites is in spite of having only three North Carolina representatives one of the most important economically.

The Effect of Periodic Illumination upon Cell Wall Structure. D. B. ANDERSON.

Potato plants were grown from a single tuber under conditions of complete darkness, constant illumination, and normal day and night. The collenchyma cells of the stem showed the same characteristic lamellation under each of these conditions. The variation in exposure to light produced no change in the structure of these cell walls.

Cotton fibers were grown under constant artificial illumination and under normal day and night conditions. Cross sections of the fibers showed no apparent differences in wall thickness. When swollen and subjected to pressure the fiber produced under outdoor conditions showed a striking lamellation. Fibers grown under constant artificial light showed no lamellation.

The wall of the cotton fiber is therefore fundamentally different in its structure and in its response to variations in environmental conditions from some other cell wall types.

Treatment of Coastal Plain Waters for Domestic and for Industrial Uses. E. E. RANDOLPH.

Surface waters of western North Carolina are as pure as any in the United States. Consequently many important industries with exacting requirements for quality of water are located in this area. The rivers rising in the mountains and flowing eastward become more highly charged with mineral and suspended matter as they approach the sea. The types of industries of the coastal plain consequently become somewhat limited. Over a period of years this investigation has been in progress to find feasible means to treat the various water supplies to make them entirely suitable for domestic and for practically all types of industries.

By inducing a larger number of wholesome industries to locate in

this area more people would be given employment and the abundant natural resources could be converted into useful products of economic value and a greater number of weekly payrolls would result in placing more money into circulation.

Much of the water of the coastal plain is suitable for certain types of industries by ordinary filtration, and by simple treatment would meet the more exacting requirements of other industries. However some of the surface and well water supplies are more difficult to condition for altogether satisfactory domestic, boiler, laundry, and special processing uses. The chemical engineer finds some real problems in some of the water supplies, but most of these problems can be economically met. The prevailing excess mineral matter consists chiefly of salts of Ca, Mg, Fe, and Na, and in some cases Mn, associated largely with bicarbonates and chlorides, with some phosphates, sulphates, and hydrogen sulphide.

Because of the abundant vegetable growth some of the surface waters contain considerable organic matter including tannic and other acids. Some of the waters are highly colored and sometimes have unpleasant odor and taste.

Near the coast sodium salts from the tides and from deep wells present serious problems.

Methods suggested for overcoming these difficulties are successive flocculation with ferrous sulphate at suitable alkalinity, accompanied by chlorination to remove color and aeration and activated carbon to remove odor and taste. To remove iron raise pH, aerate by spraying and distributing the water to present all possible surface by trickling in droplets over trays, coke, or by other means. Let settle at least four or five hours and filter.

Manganese may be removed in manner similar to iron except that pH should be higher, accompanied by chlorination and by much longer coagulation and settling period.

Since calcium and magnesium exist mainly as bicarbonates and it is desired to keep sodium salts to a minimum, lime carefully proportioned will reduce hardness to suitable limits if the water is previously aerated to remove carbon dioxide.

To overcome the brine problem from tides and from deep wells it is found that shallow wells can be sunk to yield comparatively fresh water by observing in regard to the depth the fundamental relations concerning diffusion of salt ground waters with fresh ground water near the sea coast.

The details of design and operation of any of these operations is a

major chemical engineering problem. With proper planning and operation, suitable and economic supplies of water should be possible for domestic and exacting industrial requirements.

A New Phosphorescent Species of the Genus Achromobacter. FRANK H. JOHNSON AND I. V. SHUNK.

A new species of luminous bacteria was isolated by the senior author from a dead amphipod (*Telorchestia* sp.) at Woods Hole, Mass., during the summer of 1935. It is morphologically very similar to *Achromobacter Fischeri* but may be readily distinguished from it, as well as from many other species of luminous bacteria, by virtue of the fact that neither its growth nor luminescence is favored by glycerin, and it produces no acid from this substrate in culture. Other definite physiological and cultural characteristics separate it from all reasonably adequately described species reported in the literature. The name of *Achromobacter Harveyi* has been proposed for this species.

Morphology and Hormone Production of the Testis in Relation to Age. CHARLES W. HOOKER.

In a series of 35 bulls ranging in age from one month to fifteen years the testes of each animal and a 24-hour urine specimen from each animal have been extracted for the "male sex hormone." A small portion of each of the testes was studied histologically. Assay of the testis extracts showed the hormone, present at all ages, to increase gradually, in general, to shortly after two years of age when there is a marked increase reaching a maximum at five years, after which the quantity of hormone present decreases progressively. Since the changes accompanying puberty occur in the absence of a concomitant increase in sex hormone production, the hypothesis is advanced that at puberty the tissues acquire a capacity to respond to an amount of hormone to which these tissues had previously been refractory. The diminution of hormone production which was found in the old animals indicates that senile changes may perhaps indeed be due to a withdrawal of the hormone. In marked contrast to human urine, in which the hormone is present, the hormone was absent from all these bull urines.

Histologically the young calf testis shows intertubular spaces occupied almost exclusively by mesenchymatous cells. These cells have two possible fates: connective tissue, or Leydig cells. Accompanying the completion of the development of the Leydig cells there is a marked rise in hormone production. As these Leydig cells increase in number

hormone production increases; and as these cells decrease in number in the older animals and are replaced by connective tissue, hormone production decreases. The correlation between the development of the Leydig cells and hormone production provides strong evidence that these cells are the site of production of the hormone in the testis.

Changes in the Vegetation of the Forest Floor following Elimination of Root Competition. C. F. KORSTIAN.

Whenever trees grow close enough together to form a forest stand competition occurs, that is there is a struggle between the individuals for growing space, both above and below ground, and for light, nutrients, and water. Competition results in the overtopping and crowding out of the weaker trees by the more vigorous and more aggressive ones, resulting in a natural "survival of the fittest." A tremendous struggle occurs in forests growing in moist to wet situations as they develop from very dense young stands composed of many small trees to mature forests made up of a much smaller number of large trees.

The study of the competition between the trees in a forest stand has been under way in the Duke Forest for several years. One phase of this study has involved repeated observations on 15 small areas in five different types of forest to determine the changes which take place in the vegetation of the forest floor following the elimination of root competition. In seven of the areas under observation all tree roots extending into the soil of the areas from the outside were severed by digging a trench 3 to 4 feet deep around the areas, thus eliminating the competition of tree roots for moisture and nutrients. The trenches were filled up again and detailed maps were made showing the location of all plants on each area. At least once each year thereafter a new set of maps was made. Similar maps were made for nearby untrenched areas. In this way it has been possible to follow the detailed changes in the vegetation which took place on the areas. The trenched areas have revealed very striking and almost unbelievable changes in the plant cover of the forest floor in two to three years. A great many plants have sprung up making the vegetation much more dense on the trenched than on the untrenched areas. Many additional species of plants commonly inhabiting moist areas have appeared. On many of the trenched areas, especially on the drier ones, the vegetation has become so dense and luxuriant that competition on the inside of the trenches will doubtless become intense as the tree seedlings develop. When this occurs the amount of soil moisture in the trenched areas will

probably decrease. However, determinations of soil moisture, made at intervals throughout each growing season since this experiment was begun, have thus far showed that the trenched areas contained a significantly greater amount of soil moisture than their corresponding untrenched areas every growing season since establishment. Light intensities were practically the same on the paired trenched and untrenched areas.

Evidence now available points to a very material hastening of the invasion of the trenched pine areas by hardwood trees, which commonly occur in the oak-hickory or bottomland forests of the piedmont region, due to the greater amount of soil moisture available in the trenched than in the untrenched areas. This study provides further evidence that soil moisture may often be more important than light in the death of trees in dense forests.

Inactivation of the virus of common tobacco mosaic by drying and by freezing in soil. S. G. LEHMAN.

When a field bears a crop of tobacco diseased with mosaic, the soil becomes contaminated with the virus of mosaic, but there appears to be no progressive accumulation of virus in soil with production of several successive diseased crops. The virus in the soil may be incorporated in diseased leaf, stem, or root material or it may leach from the decaying plant tissue and exist as virus particles in intimate contact with soil particles. The presence of small quantities of virus in soil may be demonstrated by appropriate methods.

When soil containing virus particles is allowed to become air dry, all or a considerable portion of the virus is inactivated and does not become active again when water is added. In drying soils which contain considerable amounts of virus, all or a large part of the virus is inactivated by the time the water content of the soil has been reduced to 4 to 5 per cent of its water-holding capacity. Freezing and thawing also inactivates tobacco mosaic virus in contact with soil particles, but is less effective quantitatively than drying.

Drying and freezing are factors which tend to prevent great accumulation of mosaic virus in tobacco soils

The Osmotic and Porous Properties of Oil Films. C. M. HECK.

Continuing the measurement reported at the 1935 meeting of the Academy of the rate of evaporation of water through thin layers of oil in freely circulating air, measurements were made in closed rooms in

which the thermograph and humidigraph showed twenty-four hour variations of less than 2°C . and 4% relative humidity. The graph of evaporation rate plotted against the thickness of oil film with thirty evaporating pans gave the same type of semiperiodic rising and falling curves that were formerly obtained in more rapidly circulating air. The conclusions were drawn that oil films in spreading tend to form into two or more types of films in which the molecular organization tends toward one form more than the other as it approaches certain thicknesses of the oil film and that the difference in the porosity due to the amount or type of organization of the molecules of the film was more effective in producing porosity than the difference in thickness.

The formerly reported discovery of the osmotic properties of thin films of mineral oils was followed up by more extended research into the method of making and testing these osmotic oil films. After soaking porous earthenware vessels in oil, drying for a few days, soaking again in gasoline, redrying, and filling with sugar solutions, the osmotic force driving water into these solutions through the vessels was found to rise in a few hours to as much as ten centimeters of mercury and fall gradually in as many days to nothing.

The Susceptibility of Stretched Rubber to Temperature Changes. MILTON L. BRAUN.

Ring-shaped rubber bands, 1 x 4 mm. in cross-section and 10 cm. in diameter, vulcanized in molds at the National Bureau of Standards, were subjected to various gravitational stresses in a controlled temperature chamber for more than 500 hours. At scattered times the temperature of the chamber was slowly raised or lowered several degrees and the resulting lengths of the bands were observed. With loads above a certain modest value the historically determined decrease in length with increase in temperature was confirmed, while for very low stresses the contrary effect of extension with increase in temperature also was confirmed. One of the chosen loads happened to lie very close to the critical value for that particular compound, and for this load, throughout temperature ranges of nearly twenty degrees, there were no observable changes in length. Most of the specimens, under their respective stresses, showed constant rates of change in length with temperature, this being true even after three weeks of drift. These constant rates for different loads were found to range from plus .00171 to minus .0550 cm. per degree centigrade. The corresponding stretches ranged from negligible to

about five times the original length of the band, the stretches being produced by loads between 10 and 1000 grams.

An appreciable time lag between the response of the bands and the response of adjacent mercury-in-glass thermometers also was observed. With one thermometer the lag behind the response of the rubber was more than a minute. Stretched rubber therefore seems to be more susceptible to temperature fluctuations than is the more sluggish mercury or alcohol thermometer, and it is conceivable that under certain local conditions a thermoscope or thermometer actuated by a piece of rubber under tension might be more serviceable than the conventional type of thermometer.

The Relation between Rate of Transpiration and Rate of Water Absorption in Plants. PAUL J. KRAMER.

The most important factor in plant water relations is really the internal water balance of the plant. This in turn is governed by the relative rates of absorption and transpiration, and the ratio of transpiration to absorption is therefore really more important than the absolute rates of the processes. Few simultaneous studies of these processes have been made because of the difficulty of measuring the absorption of plants rooted in soil. A series of such measurements were made by supplying the plant with water through Livingston auto-irrigators which permit measuring the rate of absorption of plant-plus-soil and simultaneously measuring the loss of water by weighing. The rates of absorption and transpiration were measured simultaneously at two-hour intervals over a 24-hour period for green ash, loblolly pine, sunflower, and *Opuntia*. In all four species transpiration exceeded absorption from early morning until late afternoon while absorption exceeded transpiration during the night. The maximum rate of transpiration occurred in the period from 12 to 2 p.m. in ash, pine, and sunflower, and from 4 to 6 p.m. in *Opuntia*. The maximum rate of absorption occurred two to four hours later.

The results of this experiment seem to indicate that during times of moderate or rapid transpiration plants cannot absorb water as rapidly as they lose it and a considerable saturation deficit therefore usually exists in transpiring plants during the day and even much of the night. This probably has important retarding effects on the physiological processes associated with growth. Under conditions favoring rapid transpiration and slow absorption, the amount of water absorbed at night may not equal the amount lost during the day for several days.

Such a condition results in cessation of growth and if too long continued causes death.

Auto-Narcosis in the Oyster and Its Industrial Application. H. F. PRYTHERCH.

In very young stages of *Ostrea virginica* the functioning of many internal organs can be clearly seen through the transparent shell. Soon after shell closure there is complete cessation of heart action, and movements of the blood, cilia, and digestive tract, which is accompanied by a rapid increase in the hydrogen-ion concentration of the liquor. During the first 15 minutes after closure the pH of the liquor changes from an average of 8.1 to 6.7, and gradually drops to 6.2 in oysters kept out of water for 3 to 5 days.

Experiments have shown that the tissues of the oyster are narcotized, after the shell closes, by the CO₂ generated principally by the gills, and that this condition is due primarily to the rapid development of a high CO₂ tension within the shell rather than to an increased H-ion concentration. Tests with various organic and inorganic acids show that complete narcosis cannot be produced at the pH values stated above. Carbonic acid is the exception to the rule and is particularly suitable for conserving the energy and prolonging the life of the oyster outside of its natural environment, as it readily penetrates and leaves the tissues without producing injury or noticeable after effects, as is the case with most narcotizing substances.

In commercial operations approximately 60 per cent of the oysters are shucked and wasted before marketing. It was found that the meats were rarely killed by shucking and would survive washing in fresh water up to three minutes. If then placed in the usual air-tight tin containers they would generate sufficient CO₂ to produce narcosis and reach the market in a fresh and live condition as if shipped in the shell. A small amount of "Dry Ice" or gaseous carbon dioxide can be introduced if necessary into the containers to improve the keeping qualities of the meats.

Composition of the Leaf Litter of Forest Trees. T. S. COILE.

The relatively undecomposed organic debris which characterizes the uppermost layer of the undisturbed forest soil profile is composed, for the most part, of mature leaves from the trees in the stand. Through the process of decomposition the nutritional constituents of the litter are returned to the soil where, after decomposition, they enter into the

soil solution and the exchange complex and thus become available for re-assimilation. The maintenance of forest soil fertility is contingent to a large degree on the return of the constituents of the litter to the soil and subsequent litter decomposition. The chemical composition of the litter is one of the most important factors affecting the rate and type of decomposition.

The mature undecomposed leaves of nine species of forest trees, growing on Georgeville silt loam in the Duke Forest, were analyzed for total nitrogen, carbon, calcium, and ash content. The nine species were: loblolly pine, shortleaf pine, white oak, black oak, red gum, red cedar, red maple, yellow poplar, and dogwood.

White oak leaves had the highest total nitrogen content, 1.246 per cent, and red maple leaves contained the least nitrogen, 0.497 per cent. The nitrogen content of the other species decreased regularly in the following order: red cedar, black oak, dogwood, shortleaf pine, loblolly pine, yellow poplar, and red gum. The differences between nitrogen contents of all species were statistically significant as measured by three times the standard deviation of the difference.

Differences in carbon content were not great. Loblolly pine leaves contained the most carbon, 53.15 per cent, and dogwood leaves contained the least, 48.30 per cent.

The calcium content on the basis of oven-dry weight separates the nine species into two categories: (1) those with a relatively high calcium content, 2.0 to 3.5 per cent, which includes yellow poplar, red cedar, and dogwood in increasing orders; and (2) those with a relatively low calcium content, 0.4 to 1.1 per cent, including the other species with loblolly pine containing the least.

Leaves of dogwood and white oak contain rather large amounts of ash, 7.0 to 8.0 per cent, while the pines are low in ash, 3.0 to 3.5 per cent.

The Embryo Sac of Acorus Calamus L. MURRAY F. BUELL.

In the course of development of the orthotropous ovule of *Acorus Calamus L.* two integuments are produced. Of the two-layered nucleus, the outer layer develops into a perisperm; a remnant of the inner layer remains at the base, part of it forming a "postament." Directly from the archesporial cell is produced a tetrad of megaspores, the basal one of which develops into a normal eight-nucleate embryo sac. The two synergids disappear early, and the antipodals frequently increase to as many as five. By a division of the fusion nucleus the embryo sac is

divided into a small basal and a large micropylar cell, following which fertilization takes place. The endosperm is of the basal apparatus type. The cylindrical embryo finally lies in the axis of the seed.

Geometrical Transformations. EDWARD KASNER.

The paper discusses the geometry of "Turns" and "Slides." A turn T_α is defined as the operation of turning all elements (x, y, p) through a constant angle α . A slide S_K as sliding all elements, in the direction of the element, a distance K .

A discussion is given of the 3 parameter group $T_\alpha S_K T_\beta$ called whirls. There exists a larger group, called the Turbine group, containing 15 parameters, possessing the property that a turbine is transformed into a turbine. It is pointed out that here we do not have point transformations but elements transformations, and that, in general a curve is not transformed into a curve but into a "series" of elements; a point also is transformed into a series (American Journal of Mathematics, 1916).

The work is then generalized to the theory of isogonal series and equitangential series. We are thus led to new groups related to conformal and equilong transformations.

Some Remarks on Stieltjes Integrals. F. G. DRESSSEL.

The paper discusses the interrelations of the Young- and Radon-Stieltjes integrals. It concludes with the theorem

$$\int_0^1 l(y) df(y) = \int_0^1 l(y) Df dg(y)$$

where Df is the derivative of the function of limited variation $f(y)$ taken with respect to the monotone increasing function $g(y)$, and $l(y)$ is bounded and Borel measurable.

Some Theorems on Tensor Differential Invariants. J. LEVINE.

In this paper it is shown that in a relation of the form

$$T_{c \dots d}^{a \dots b} \left(\bar{g}_{ij}; \dots; \frac{\partial^m \bar{g}_{ij}}{\partial x^{k_1} \dots \partial x^{k_m}} \right) u_a^p \dots u_b^q = \phi(u) T_{r \dots s}^{p \dots q} \left(g_{ij}; \dots; \frac{\partial^m g_{ij}}{\partial x^{k_1} \dots \partial x^{k_m}} \right) \cdot u_c^r \dots u_d^s$$

where g_{ij} defines the metric of a metric space and $u_j^i = \frac{\partial x^i}{\partial \bar{x}^j}$. The $\phi(u)$ is a power of the Jacobian of the coördinate transformation. The proof is based on the partial differential equations which tensor invariants satisfy, these equations being found in a paper by T. Y. Thomas and A. D. Michal, *Annals of Mathematics* (1927).

On the Equations of Conics of r-point Contact. J. W. LASLEY, JR.

The general equation of the conic together with the first five equations obtained by differentiation with respect to x give us a set of equations, the last three of which have as their resultant Monge's differential equations. If the fourth and fifth of these equations are solved for certain ratios, and the results placed in the first three, we obtain expressions for the coefficients of the general equation of the second degree as differential forms involving x, y, p, q, r , and s , where each of the foregoing is the derivative of the preceding as to x . If now we merely replace these quantities by correspondingly formed quantities x, Y, P, Q, R , and S for any point of a general curve, we obtain the equation of the conic of 5-point contact at that point. If we replace x, y, p, q , and r by x, Y, P, Q , and R , leaving s free, we obtain the equation of the pencil of penosculating, or 4-point conics. Continuing in this way we are able to obtain all conics of r -point contact for $r = 5$, or less.

Algebraic Systems. J. M. THOMAS.

The paper develops a new method for solving systems of simultaneous algebraic equations. It employs the resultant of two polynomials to give a reduction algorithm by means of which any algebraic system S of equations and inequations is factored in the form $S = T_1 T_2 \dots T_l$, where the T 's are *simple systems*, having special properties. In particular, (1) the fundamental theorem of algebra can be applied to each factor T ; (2) the roots of each T are simple; and (3) there is an easy test for equivalence of systems when they are resolved into simple factors.

The factorization into simple factors is not unique. *Prime systems* are defined. The resolution into prime systems is essentially unique, but there is no algorithm for effecting the factorization, which in particular requires finding the irreducible factors of a polynomial.

The features of the present treatment which seem to be especially advantageous are (1) the systematic discussion of inequations along with equations; (2) the elimination of multiple roots; and (3) the applicability of the reduction algorithm without previous linear transformation of the indeterminates.

The Effects of Acids and Bases upon the Infra-red Absorption Bands of Water. E. K. PLYLER AND E. S. BARR.

Previous studies have shown that there are present absorption bands in solutions of acids and bases which are characteristic of the ions that are present. Also bands are produced by the undissociated molecules. In this study attention has been placed upon the change in intensity

and position of the water bands as produced by the presence of the acids and bases. It has been found that the intensity of the bands due to water is increased by the presence of the acids and bases. Also there is usually a shift in position of the water bands when measured in solutions.

Heat Transfer across an Interface Between Oil and Water. C. M. HECK
AND G. W. BARTLETT.

The heat flow in each direction across an oil-water interface was found to be associated with a sudden difference in temperature at the interface amounting to about two degrees for the conditions in two designs of apparatus used. In one case the heat was applied at the bottom of a tall cylinder half filled with oil and half with water; temperature gradients were read throughout the whole length of both liquids and across the interface. In the other a short cylinder closed by a steam reservoir at the top and a cold water-circulating reservoir at the bottom had a set of opposed thermocouples, one as much above the interface as the other was below, rotated about an axis of support in the interface. The difference in temperatures of the two junctions were plotted against their distance apart as they approached the interface. Each quadrant of a complete revolution produces a curve extending from a projection onto the zero-drop axis to a maximum value. These curves are straight lines in pairs displaced apart about five degrees in temperature with the two in each pair practically coincident. In each liquid extrapolation of the temperature gradients back to the interface indicates in each of the above types of apparatus a discontinuity of temperature which discontinuity value is inversely related to the amount of disturbance at the interface by convection and agitation. The discontinuity indicated is negative when the direction of heat flow is from oil to water and positive when the flow is in the opposite direction.

PROCEEDINGS OF THE ELISHA MITCHELL SCIENTIFIC SOCIETY

OCTOBER 8, 1935, TO MAY 12, 1936

362ND MEETING, OCTOBER 8, 1935

W. C. DAVISON: *Medical Problems Peculiar to North Carolina.*

A number of diseases common in North Carolina were described. These included "blue gum negro bite," caused by spirochetes and curable by arsphenamine, fungus infections caused by blastomycetes or actinomycetes and curable in most cases by the administration of iodides, and lung abscesses following amebic dysentery. With regard to pellagra, it is now believed to be a resultant of three factors: (1) a dietary deficiency; (2) exposure to sunlight; (3) intestinal infections. North Carolina is second to Alabama in the incidence of pellagra and it is most prevalent in the month of June in this state.

The treatment of arthritis by artificial inducement of fever was described and the question was raised whether fever may not be nature's method of combatting a diseased condition.

The use of the ketogenic diet for the treatment of epilepsy was discussed and it was stated that this treatment causes improvement in 50-75 per cent of the cases.

The recent epidemic of poliomyelitis was discussed. This epidemic was unusual in that it reached its peak in early June although most previous epidemics reached their peak in September.

In conclusion some remarks were made on intestinal parasites, empyema, anemia, Malta fever, and infant mortality.

363RD MEETING, NOVEMBER 12, 1935

G. R. MACCARTHY: *Magnetic Anomalies and Geologic Structures on the Carolina Coastal Plain.*

A magnetically disturbed zone, roughly parallel to the coast, has been outlined on both sides of the North-South Carolina border. This zone consists of a number of sub-parallel strips of alternately high and low intensity in the vertical component of the earth's magnetic field. It has been traced from the vicinity of Myrtle Beach to Wilmington,

and apparently continues further in a northeasterly direction. This disturbed zone probably represents a folded and perhaps a fractured belt in the crystalline basement rocks which are buried under about 2000 feet of Coastal Plain sediments in this region, and seems to be very similar to the disturbed zone which has been reported from the Florida peninsula.

The complete paper will appear in an early issue of the "Journal of Geology."

EDWARD MACK, JR.: *The Size and Shape of Molecules.*

Chemists and physicists have speculated about the existence of particles of matter for a very long time, and have used the idea of the atom and the molecule in their thinking and theorizing, even though there was no very definite evidence for the objective existence of such particles. Within the past fifty years, however, a great mass of rather convincing evidence has been accumulating, which leads to the view that atoms and molecules actually do exist. Indeed it is possible to deduce, by using certain physical methods, the sizes and the shapes of atoms and molecules.

The most powerful tool, so far developed, for determining the dimensions of these particles and something about their shapes is the method of x-ray analysis. (Here was given a brief description of the physical principle involved, and several scaled models were shown of the crystal lattices of sodium chloride, copper, iron, iodine, diamond, graphite and benzene.)

Another approach to size and shape, especially in the case of long hydrocarbon chain molecules with a polar, water-soluble, group at one end of the molecule, is the oil-film method developed in this country largely through the genius of Dr. Langmuir. (Here a blackboard description was given of the orientaton of such stick-like molecules on a water surface, and the method was presented for estimating the cross-section and the length of the molecules from the area and thickness of the oil-films.)

Of the half-dozen other methods which may be employed, one further method was described, namely the approach through measurement of the rate of diffusion of a volatile substance into air or the rate of viscous flow of a volatile substance down a capillary tube. From diffusion coefficients or from viscosity coefficients (at several temperatures) the collision areas of the molecules may be calculated. By matching these

areas against the average shadow areas of models, made to scale, definite conclusions about size and shape of the molecules may be reached.

364TH MEETING, DECEMBER 10, 1935

T. P. NOE, JR.: *The Determination of Stress in an Elastic Body.*

The principle of least work, developed by Castigliano, states that the internal work done in any structure by the application of outer forces will be the least possible, consistent with equilibrium. The application of this principle to the solution of statically indeterminate reactions and stresses in redundant members of trusses is quite familiar to engineers. However, the principle may also be used as a criterion for selecting from a number of possible cases the most probable distribution of stress intensity over a given cross section. The truth of the result obtained will depend upon whether the correct distribution has been included in the group tested.

As an illustration, the distribution of stress in a curved beam, subjected to pure bending, will be considered. For this case there are two variations of stress intensity that have been advanced and used by engineers: namely, that of linear variation (planar stress) and that of hyperbolic variation (planar strain). The two assumptions yield quite different results and only recently has it been determined by experiment that the hyperbolic variation is correct. The same result may be obtained by comparing the value of the total internal work computed on the basis of the two proposed distributions, the hyperbolic variation producing the least value for the internal work when the curved beam is subjected to a known external loading.

The fact that the internal work serves so well as a selective process is all the more interesting because the value of the internal work varies but slightly as the type of stress distribution considered is changed radically.

JOHN A. WHEELER: *The Formation of the Chemical Elements.*

Chemical analysis of samples of the earth's crust, of meteorites, and spectrochemical studies of the atmospheres of the sun and other stars give evidence that on the whole the earth is similar in its composition to the universe as a whole. Figures kindly supplied by Professor G. von Hevesy lead to the following estimates of the abundance of the more common isotopes in the solar system:

$\left. \begin{smallmatrix} 16 \\ 8 \end{smallmatrix} \right\} \text{O} \dots\dots\dots 52\%$	$\left. \begin{smallmatrix} 29 \\ 14 \end{smallmatrix} \right\} \text{Si} \dots\dots\dots 0.8\%$
$\left. \begin{smallmatrix} 23 \\ 11 \end{smallmatrix} \right\} \text{Na} \dots\dots\dots 0.6$	$\left. \begin{smallmatrix} 30 \\ 14 \end{smallmatrix} \right\} \text{Si} \dots\dots\dots 0.8$
$\left. \begin{smallmatrix} 24 \\ 12 \end{smallmatrix} \right\} \text{Mg} \dots\dots\dots 10$	$\left. \begin{smallmatrix} 32 \\ 16 \end{smallmatrix} \right\} \text{S} \dots\dots\dots 2.6$
$\left. \begin{smallmatrix} 25 \\ 12 \end{smallmatrix} \right\} \text{Mg} \dots\dots\dots 1.3$	$\left. \begin{smallmatrix} 40 \\ 20 \end{smallmatrix} \right\} \text{Ca} \dots\dots\dots 0.9$
$\left. \begin{smallmatrix} 26 \\ 12 \end{smallmatrix} \right\} \text{Mg} \dots\dots\dots 1.3$	$\left. \begin{smallmatrix} 54 \\ 26 \end{smallmatrix} \right\} \text{Fe} \dots\dots\dots 0.6$
$\left. \begin{smallmatrix} 28 \\ 14 \end{smallmatrix} \right\} \text{Si} \dots\dots\dots 12$	$\left. \begin{smallmatrix} 54 \\ 26 \end{smallmatrix} \right\} \text{Fe} \dots\dots\dots 12$

Heavy atoms, in comparison, are relatively rare; for example, only 0.0000026% of all atoms are estimated to belong to the species $^{209}_{83}\text{Bi}$.

Experiments have shown in the past few years that fast charged particles (protons, ^1_1H ; deuterons, ^2_1H ; and alpha particles, ^4_2He) are capable in close collisions of transmuting the lighter nuclei into heavier ones, often with the release of much energy. Objections to an explanation of element building on this basis are two in number: it has not proved possible in the laboratory to transmute the elements of medium and high atomic weight in this way; and at stellar temperatures, of the order of 10,000,000 degrees absolute, the charged particles are moving with an energy a hundred thousand times too small to be effective in collisions with light nuclei.

Promise of at least a partial accounting for the formation and relative abundance of the chemical elements comes from experiments of Enrico Fermi in 1934-35. Slow neutrons were found strikingly effective in transmutations of the type $^1_0\text{n} + ^{127}_{53}\text{I} \rightarrow ^{128}_{53}\text{I}$, where the heavy isotope formed is unstable and disintegrates, giving an element of atomic number one unit higher, $^{128}_{54}\text{Xe}$, and an electron. So efficient are neutrons in producing nuclear changes that the presence of only a small proportion of them within a star would very quickly give rise to an extremely large liberation of heat, through the transmutations they bring about. Spectrochemistry does not tell whether neutrons are present in the stars. We may hope in the next few years to learn the answer to this question, and to trace in detail the successive changes through which the heaviest elements are built up from the lightest.

365TH MEETING, JANUARY 14, 1936

(Joint meeting with the Philological Club)

J. O. BAILEY (English): *Science—Preserver or Destroyer? Some Literary Futurities.*

366TH MEETING, FEBRUARY 11, 1936

R. W. BOST: *A Decade of Organic Chemistry.*

A review of some of the most important developments in organic chemistry during the last decade was given. The following topics were discussed: vitamins, hormones, anesthetics, disinfectants, perfumes, detergents, cracking of hydrocarbons, synthetic fuels, refrigerants, plant stimulants, synthetic resins, plant pigments and insecticides.

367TH MEETING, MARCH 3, 1936

E. W. MCCHESENEY: *The Analysis of Proteins.*

The proteins are perhaps the most important compounds found in living matter, and it is essential to the progress of biological chemistry that their structures and composition should be elucidated. In 1820 Braconnot discovered the first amino acid to be found in a protein; namely, glycine. The discovery of others followed rather rapidly until it became apparent about 1900 that proteins were made up entirely of alpha-amino acids. This led Fischer and Hofmeister to propose the "peptide" hypothesis of protein structure, which remains the most satisfactory conception. The recent work of Astbury on the X-ray diagrams of proteins tends to lend further support to the view.

Although accurate methods have been devised for the determination of some of the amino acids, our knowledge of the composition of many of the proteins is far from complete, ten to forty percent usually being unaccounted for. The remainder may represent undiscovered amino acids or analytical inaccuracies.

A fruitful method of study has been the separation of groups of amino acids having similar properties. A good example is the method of Foreman for the dicarboxylic amino acids. This aided Dakin in his discovery of hydroxyglutamic acid. In an attempt to repeat Dakin's work, the author found that casein yields a large fraction of amino acids which have water-insoluble barium salts. The principal component of this fraction is glutamic acid, but hydroxyglutamic acid is present and aspartic acid is absent. In addition a compound precipitable by phosphotungstic acid is present and it appears to be different from anything

previously described. It is not one of the diamino acids, and gives a strong diazo reaction. Further work with a view toward determining its nature is in progress.

DONALD P. COSTELLO: *Some Effects of Centrifugal Force on Marine Eggs.*

During the past 30 years the centrifuge has proven to be an instrument of extraordinary delicacy for bringing about a new distribution of the formed materials in certain living cells. The egg cells of many animals after being subjected to such treatment show a characteristic distribution of the fat, the yolk, and the pigment. The forced movement of these components through the egg, displacing the protoplasm, does not seem to injure the living substance, nor impair greatly its capacity for development. By the use of the centrifuge it has been possible to study many problems connected with the possible rôle of certain substances which might be thought to determine the future course of development.

The centrifuge used was an air-turbine ultracentrifuge of the Beams type, which gave forces up to 270,000 times gravity. Some unfertilized eggs of the marine worm, *Nereis*, centrifuged at 250,000 times gravity for 15 minutes, or at 100,000 times gravity for 60 minutes, subsequently developed, apparently normally.

The accumulation of vital dyes in particular formed cell components, rather than their coloration of the hyaline protoplasm, has also been demonstrated by the use of these high centrifugal forces. This indicates that the methods of measurement of the hydrogen ion concentration of hyaline protoplasm, employing such dyes, are completely inadequate.

Other problems related to the formation of the fertilization membrane have been studied in eggs of other marine forms using these high centrifugal forces.

368TH MEETING, APRIL 14, 1936

J. N. COUCH: *Some Recent Advances in Botany.*

369TH MEETING, MAY 12, 1936

H. M. BURLAGE: *The Development of the United States Pharmacopoeia and Formulary.*

Beginning with the Period of the Egyptian Papyri (previous to 1000 B. C.) works dealing with medicines and their preparations were discussed. From 1000 B. C. to 1000 A. D. was the period of works of individuals; 1000 A. D. to 1500, the period of Antidotariums; 1500 to 1800, City Pharmacopoeias; and 1800 to date, National Pharmacopoe-

ias. The development of the U. S. Pharmacopoeia was discussed through the present eleventh revision, with emphasis on the work of revision, the pharmacopoeial conventions and their compositions, important changes and trends and a comparison of these changes, trends and contents with other National Pharmacopoeias was discussed.

The National Formulary—previously called the National Formulary of Unofficial Preparations and a companion work of the U. S. Pharmacopoeia—is a product of the American Pharmaceutical Association. The ways and means of its development and revision were discussed. The relationship of these two national works in the enforcement of the Pure Foods and Drug Act was emphasized.

The following officers were elected for the year 1936-1937:

President—R. W. Bost.

Vice-President—English Bagby.

Secretary-Treasurer—E. L. Mackie.

The permanent secretary, E. T. Browne, and the editors of the Journal, W. C. Coker, H. V. Wilson, and Otto Stuhlman, continue in office.

ESTUARINE ANIMALS AT BEAUFORT, NORTH CAROLINA

By A. S. PEARSE

PLATES 15 AND 16

INTRODUCTION

Beaufort is very favorably situated for the study of estuarine animals. It is near the open sea, but protected by Shackleford and Bogue Banks; it is surrounded by sounds, flats, marshes, and estuaries. Naturalists have long visited Beaufort. As early as 1860 Gill and Stimpson worked there; Coues and Yarrow were stationed at Fort Macon and Bogue Bank in 1871-1872 and published a list of animals that they found. In 1902 the present laboratory building of the United States Bureau of Fisheries was opened on Piver's Island. During the three preceding years the Bureau had maintained a laboratory in temporary quarters within the town of Beaufort. Since 1880, when the well known work of the marine laboratory of the Johns Hopkins University under Professor W. K. Brooks began, many scientific men have carried on investigations, and the flora, fauna, and hydrography are therefore well known.

The writer first worked at Beaufort during the summer of 1928. Grateful acknowledgment is made to Miss Sophie Dehler who helped with field and laboratory work at that time. In the spring and summer of 1934 Mr. Henry Hatsell gave excellent service as assistant and Miss Elizabeth Huntley helped during part of the summer. Dr. S. F. Hildebrand and Dr. H. F. Prytherch, Directors of the Beaufort Biological Station, United States Bureau of Fisheries, Captain Charles Hatsell and other members of the staff at the station did everything possible to help make the work successful. Many specialists have kindly identified specimens. For such gratuitous services acknowledgment is made as follows: plants, H. L. Blomquist, H. J. Oosting; foraminiferans, J. A. Cushman; trematodes, E. W. Price; nematodes, B. G. Chitwood; polychaetes, A. L. Treadwell; leeches, J. P. Moore; echinoderms, H. L. Clark; molluscs, Henry van der Schalie, P. Bartsch; copepods, C. B. Wilson; ostracods, Norma C. Furtos; barnacles, J. P. Visscher; amphipods, C. R. Shoemaker; isopods, J. O. Moloney; malacostracans, Mary

J. Rathbun, W. L. Schmitt; mites, Ruth Marshall; spiders, A. M. Chickering; insects, C. S. Brimley; collembolans, J. W. Folsom; odonates, P. P. Calvert, Jay R. Traver; bugs, H. B. Hungerford; orthopterans, A. N. Caudell; beetles, H. S. Barber, A. G. Bovig, L. L. Buchanan, W. S. Fisher, H. Morrison, C. F. W. Muesebeck, P. W. Oman; dipterans, H. G. Barber, C. T. Greene, G. A. Sandhouse, A. Stone. Mr. Richard Bellaire rendered good service in entering records.

In 1928 considerable time was spent in testing the ability of various animals to live when transferred to sea water or dilutions of it, but some collecting was done. In 1934 most of the time was devoted to collecting specimens and samples. In both years observations were made at twenty-two stations and in 1934 determinations of salinity, oxygen, hydrogen-ion concentration, and temperature were made continually.

STATIONS STUDIED

The following list gives the localities where observations were made and where collecting was done.

1. Shackleford Bank, beach on open sea.
2. Fort Macon; flats and small lagoon on Bogue Sound.
3. Shackleford Bank, beach on Back Sound.
4. Breakwater north of Fort Macon.
5. Mullet Pond, Shackleford Bank.
- 5A. Cattail Swamp, East of Mullet Pond.
6. Bogue Sound.
7. Bird Island Shoal.
8. Town Marsh.
9. Piver's Island.
10. Lenoxville Road, ditches and muddy shore.
11. Calico Creek.
12. John L. Godett's Sound.
13. North River.
14. Newport River.
15. Core Creek.
16. Eastman's Creek, off Core Creek.
17. Adam's Creek and Core Creek Canal.
18. Harlowe Creek.
19. Morton's Mill Pond.
20. Creek above Morton's Mill Pond.
21. Open Grounds.
22. Carteret Lodge Pond.

VARIATIONS IN PHYSICAL FACTORS

The stations just listed range from the open ocean to freshwater streams and ponds where salt-water never enters, and which lie twenty miles from the coast. When onshore winds prevail for several days salt water may back up into the waters of all the stations studied except the last three (20, 21, 22). On the other hand with prolonged offshore winds the waters of streams near the coast (11, 16, 18, 19) may remain quite fresh. Mullet Pond (5) is of interest because it lies on a narrow (0.5 mile) bank covered with moving sand dunes, and is close to the open sea. Its salinity varies greatly (0.04–26.94 ‰). When there are no high tides for a long period the little swamp (5A) and stream to the east feed nearly fresh water (S:0.30–0.84 ‰) into it; when high tides inundate the flats below it the water becomes quite salty. Temperatures of course vary with seasons and in severe winters ice forms in rather saline waters, but most marine and freshwater animals in the region studied escape freezing by burrowing into sandy or muddy bottoms. Hydrogen-ion concentrations also vary because of winds, floods, temperature, changes in salinity, depth, and other factors. The following observations show this:

Carteret Lodge Pond, June 28, 10:00 A.M.

Surface, S 0.05 ‰, T 31.1°C., pH 6.2.

Carteret Lodge Pond, June 28, 12:00 A.M.

Surface: S 0.05 ‰, T 32.5°C., pH 6.4, O₂ 3.43.

Bottom, 2 ft.: S 0.05 ‰, T 29.0°C., pH 6.3, O₂ 2.09.

Bottom, 6 ft.: S 0.05 ‰, T 26.9°C., pH 6.2, O₂ 1.48.

Stream from Open Grounds to North River, July 3.

Mouth

Surface: S 20.10 ‰, T 30.4°C., pH 6.8, O₂ 5.18.

Bottom: S 21.36 ‰, T 31.2°C., pH 6.8, O₂ 5.19.

Middle

Surface: S 0.07 ‰, T 28.5°C., pH 7.4, O₂ 3.02.

Bottom: S 0.07 ‰, T 27.3°C., pH 7.2, O₂ 2.92.

Headwaters; depth, 4 in.

S 0.07 ‰, T 30.0°C.

Mullet Pond, July 11.

Surface: S 26.10 ‰, T 27.3°C., pH 8.6+.

Bottom: S 24.92 ‰, T 27.0°C., pH 8.6+.

Morton's Mill Pond, July 5.

Surface: 20.0 ‰, 33.8°C., pH 8.4, O₂ 8.87.

Bottom: 20.8 ‰, 32.1°C., pH 8.2, O₂ 6.39.

Oxygen was usually present in considerable quantities but the data just given show that it was at times somewhat lower near the bottoms of small ponds and streams than at the surface. Carter (1934) found similar conditions in British Guiana. The writer's figures represent cubic centimeters per liter.

Tides at Beaufort normally have a range of 2 ft. 7 in., but spring tides are about 3 ft. 6 in. and during great storms tides may reach 5 or 6 feet.

LIST OF ANIMALS OBSERVED

Many species in the following list are not in the card catalogue of the Beaufort fauna maintained at the Beaufort Biological Station, United States Bureau of Fisheries, and many there listed have not been seen by the writer. The two lists should therefore supplement one another. When the card catalogue contains information as to general distribution of a species studied by the writer at Beaufort, it is appended to species mentioned in this list.

Protozoa

Foraminiferida

Elphidium poeyanum (d'Orbigny)

Mullet Pond, in tow net; July 11, 1935.

Rotalia beccarii (L.) *parkinsonia* (d'Orbigny)

Morton's Mill Pond (S:8.95), May 1. Mullet Pond, June 26, (S:3.60), July 11 (S:3.20, 0.3); Carteret Lodge Pond (S:0.05), June 28, 1934.

Porifera

George and Wilson (1921) describe 17 species of sponges from Beaufort Harbor and vicinity. None of these was identified during the present study.

Coelenterata

Hydrozoa

Cordylophora lacustris Allman

Open Grounds; ditch (S:8.73), June 28, 1928.

Eudendrium carneum Clarke

Core Creek on *Libinia* carapace; June 16, 1928.

Hydractinia echinata Fleming

Piver's Island; June 13, 1928; on hermit crab's shell.

Physalia pelagica (L.)

Beaufort Inlet; July 23, 1928.

Tubularia crocea Agassiz

Piver's Island; August 13, 1928.

Nemopsis bachei Agassiz

Morton's Mill Pond (S:8.95); May 1, 1934. Several in tow, May, 1914.

Scyphozoea

Dactylometra quinquecirrha (Desor)

Morton's Mill Pond (S:20.4); July 5, 1934.

Anthozoea

Gorgonia verrucosa Agassiz

Piver's Island; August 10, 1928; Bogue Sound, March 30, 1934.

Edwardsia sp.

Young anemones of this genus were found, Mullet Pond, April 30 (S:9.11) and June 18 (S:12.54); Town Marsh, June 27.

Sagartia luciae Verrill

Core Creek Canal, June 14, 1928; Breakwater north of Fort Macon, June 25, 1928.

Astrangia danae Agassiz

Piver's Island, June 13, 1928; Bogue Sound, March 30, 1934.

Ammophilactis rapiformis Verrill

Sheep's Head Shoal; June 14, 1928. Cast up on Fort Macon beach after storm.

Ctenophora

Mnemiopsis leidyi Agassiz

Sheep's Head Shoal; June 15, 1928. Piver's Island, March 31; Morton's Mill Pond, June 19 (S:8.95), May 1 (S:5.0); Harlowe Creek, June 22 (S:3.25); Mullet Pond (S:3.70); June 26, 1934. Often common.

Platyhelmintha

Turbellarea

Bdelloura candida (Girard)

On *Limulus polyphemus* (L.) Breakwater Flat, June 13, 1928; Bogue Sound, March 31; Town Marsh, June 27, 1934.

Trematodea

Levinseniella jagerskioldi Travassos

Piver's Island; April 1, 1934, in *Uca pugilator* (Bosc).

Nemertea

Cerebratulus lacteus Leidy

Core Creek; June 14, 1928. Under stones on Shackleford Bank.

Nemata

Acuarine larvae

In visceral cysts in *Uca minax* (Le Conte), Harlowe Creek, April 3, 1934.

Metaparoncholaimus sp.

Mullet Pond, July 11, 1934 (S:19.8).

Spilophorella sp.

Mullet Pond, July 11, 1934 (S:19.8).

Bryozoa

Bugula turrita (Desor)

Piver's Island, June 12, 1928.

Schizoporella unicornis (Johnston)

Piver's Island; June 12, 1928.

Echinodermata

Asterea

Asterias forbesi (Desor)

Town Marsh; June 13, 1928.

Ophiurea

Ophiothrix angulata Say

Piles on bridge; June 12, 1928. Common.

Ophioderma brevispina (Say)

Bird Island Shoal, August 13, 1928; Bogue Sound, March 30, 1934.
Common.

Echinoidea

Arbacia punctulata (Lamarck)

Piver's Island, June 12, 1928. Common.

Mellita quinquiesperforata (Leske)

Breakwater Flat, June 13, 1928; Bogue Sound, March 30; Bird Island Shoal, June 25, 1934. Abundant on suitable bottoms along open sea.

Holothurea

Thyone briareus (Lesueur)

Sheep's Head Shoal, June 15, 1928. Rather common.

Leptosynapta inhaerens (O. F. Müller)

Breakwater Flat; June 13, 1928. Not very common; larvae at times abundant in towings.

Annelida

Polychaetea

Autolytus varians Verrill

Core Creek Inlet; June 16, 1928.

Nereis limbata Ehlers

Breakwater Flat, June 13, 1928; Morton's Mill Pond, April 3 (S: 2.48), June 19 (S:5.6); Calico Creek, May 2 (S:16.5); July 6 (S:32.4); Core Creek Canal, July 10 (S:3.55), 1934.

Leptonereis culveri Treadwell

Mullet Pond, June 18 (S:13.0), July 11 (S:3.2, 19.8), 1934.

Nephtys buccera Ehlers

Piver's Island, June 12, 1928.

Onuphis magna Andrews

Bogue Sound, March 30, 1934 (S:27.77). Common on Bird Island and Shark Shoals.

Onuphis cuprea (Bosc)

Breakwater Flat; June 13, 1928. Piver's Island.

Glycera americana Leidy

Piver's Island; June 13, 1928; Mullet Pond, June 26, 1934 (S:12.6).

Chaetopterus pergamentaceus Cuvier

Breakwater Flat, June 13, 1928. Common in appropriate places.

Amphitrite ornata (Leidy)

Core Creek, June 14, 1928. Common.

Hydroides hexagonus Bosc

Piver's island; June 12, 1928. Common on old shells.

Hirudinea

Placobdella rugosa (Verrill)

Carteret Lodge Pond, June 28, 1934; S:0.05.

Myxobdella lugubris Leidy

Mullet Pond, April 1, June 26, July 11, 1934; common on *Callinectes sapidus* Rathbun.

Piscicolaria, juv.

Echiurea

Thalassema mellita Conn

Breakwater Flat; June 13, 1928.

Mollusca

During 1933 Dr. Henry van der Schalie, Museum of Zoology, University of Michigan, worked at the Beaufort Biological Station. He has furnished a list of molluscs identified by him and Dr. W. J. Clench. These are preceded by a star(*) in the list. Other specimens were referred to Dr. van der Schalie for identification. Jacot (1921) published a list of the marine molluscs of Beaufort and reviewed the work of previous writers.

Gastropodea

**Diadora alternata alternata* Say

Breakwater Flat, June 13, 1928. Common on trestle near Morehead City.

**Turbo castaneus castaneus* Gmelin

Town Marsh, July 18, 1928.

Melanella conoidea* Kurtz & StimpsonLittoridina monroensis* (Frd.)

Calico Creek, May 1 (S:8.95), June 21 (S:29.2); Morton's Mill Pond, June 19 (S:4.9); Mullet Pond, June 18, (S:12.54), June 26 (S:12.6); July 11, 1934 (S:19.8).

Pyramidella crenulata* HolmesNatica pusilla* Say**Sinum perspectivum* Say

Bird Island Shoal, August 10, 1928. Common on Bird Island Shoal.

**Polynices duplicata* Say

Piver's Island, June 12, 1928. Common in Beaufort Harbor.

Calyptraea centralis* ConradCrepidula fornicata* (Verrill)

Piver's Island, June 12, 1928; Mullet Pond, June 18, 1934. Common on *Limulus* and shells.

**Crepidula plana* Say

Piver's Island, June 12, 1928; Bogue Sound on *Leniulus*, March 31, 1934. Common on *Limulus*, shells of hermit crabs, etc.

Paludestrina tenuipes Couper

Above Morton's Mill Pond, May 1 (S:0.73); Calico Creek, May 2, 1934 (S:0.76).

**Littorina irrorata* Say

River's Island, June 12, 1928; Calico Creek, May 12 (S:16.52), June 21 (S:4.69), July 6 (34.1); John Godett Bay, June 19 (S:13.85); Core Creek Canal, July 10 (S:0.7, pH 6.8); July 12, 1934 (S:15.52). Common on sedges.

**Epitonium lumphreysii* Keiner

On drift from Shark Shoal.

Triphora perversa nigrocincta* C. B. AdamsSeila adamsii* H. C. Lea**Strombus pugilis pugilis* L.

At times taken in seines outside the banks.

**Simnia uniplicata* Sowerby.

On piles and gorgonian corals.

Cassis tuberosa* (L.)Cassis inflata inflata* Shaw**Tonna galea* L.**Ficus papyratia* Say**Eupleura caudata caudata* Say

Piver's Island, June 12, 1928.

**Urosalpinx cinereus* (Say)

Piver's Island; June 12, 1928. Common on piles and breakwater at Shackleford Bank and Fort Macon.

**Thais floridana floridana* Conrad

On Morehead trestle and Shackleford Bank.

Anachis avara avara* (Say)Anachis obesa obesa* C. B. Adams

Common in Bogue Sound.

Mitrella lunata lunata* SayNassarius vibex* (Say)

Piver's Island, June 12, 1928. Common on Shark Shoal.

**Nassarius obsoleta* (Say)

Piver's Island, June 12, 1928. Abundant on mud flats generally.

Nassarius trivittata* (Say)Busycon carica carica* Gmelin

Abundant on Bird Island and Shark Shoals.

**Busycon perversum perversum* (L.)

Reported by van der Schalie and Clench. Common on Bird Island and Shark Shoals and near Fort Macon Beach.

**Fasciolaria distans* Lamarck

Piver's Island, June 12, 1928. Common on mud flats near Piver's Island, Shark Shoal, etc.

Marginella apicina apicina* MenkeOliva sayana sayana* Ravenel

Piver's Island, June 12; Town Marsh, June 13, 1928. On Shark and Bird Island Shoals; on sandy beaches.

**Olivella mutica* Say

Breakwater Flat, June 13, 1928. On Shark and Bird Island Shoals.

**Terebra dislocata dislocata* Say

Breakwater Flat, June 13, 1928. Common on shoals.

Mangilia cerina* Kurtz & StimpsonMangilia plicosa* C. B. Adams**Acteocina canaliculata* Say*Elysia chlorotica* Gould

Morton's Mill Pond, April 3, 1934.

Polygyra thyroides (Say)

Harlowe Creek, April 4, 1934.

Philomycus carolinensis (Bosc)

Open Grounds, ditch, July 2, 1934.

Succinea campestris Say

Open Grounds, ditch, July 2, 1934.

**Melampus lineatus* Say

Mullet Pond, June 22; North River, June 29, 1928; Harlowe Creek, April 2, June 22; Calico Creek, April 2; Open Grounds, July 2; Eastman's Creek, July 12, 1934.

Lymnaea columella Say

Open Grounds, June 20 (S:0.3); Carteret Lodge Pond, June 28, 1934 (S:0.05).

Physa pomilia hendersoni Clench

Carteret Lodge Pond, June 28, 1934 (S:0.05).

Gyraulus dilatus Gould

Open Grounds, ditch, June 20, 1934 (S:0.3).

Ferrissia sp.

Open Grounds, ditch June 20, 1934 (S:0.3).

Pelecypodea

**Solemya velum* Say

Piver's Island, June 12, 1928. Common on shoals.

Nucula proxima proxima* Sowerby*Nuculana acuta* (Conrad)Arca campechiensis* Dillwyn**Arca incongrua* Say**Arca occidentalis* Philippi**Arca secticostata* Reeve**Arca transversa* Say

Abundant half a foot below low-water mark on muddy bottoms among eel grass; Horse Island. This is the commonest representative of the genus.

**Noetia ponderosa* Say

Breakwater Flat, June 13, 1928. On muddy bottoms; less common than last species.

**Eulima conoidea* Kurtz & Stimpson

In mud among dead shells; fairly common.

Glycimeris pectinata* GmelinPanopea floridana* Heilprin**Atrina rigida* (Dillwyn)

Piver's Island, June 12, 1928. On muddy bottoms. Rather common.

Atrina serrata* SowerbyPteria eximia* Reeve

On gorgonian corals.

**Ostrea virginica* Gmelin

Newport River, June 29, 1928 (S:35.95); Adams Creek, April 2 (S:0.0); Harlowe Creek, June 22 (S:0.5), July 5 (S:20.0). Eastman's Creek, July 12, 1934 (S:15.5). Alongshore, largely found from a foot below tide mark upward.

**Ostrea equestris* Say

Found mostly from low-tide mark to depth of about ten feet.

Plicatula gibbosa* LamarckPecten irradians irradians* Lamarck

Piver's Island, June 12, 1928. Bogue Sound, June, 1934. This is the common scallop among eel grass.

**Pecten gibbus gibbus* L.

By some thought to be synonymous with the last species.

**Anomida simplex* d'Orbigny

Breakwater Flat, June 13, 1928. Common on old shells.

**Mytilus exustus* L.

Breakwater Flat, June 13, 1928; Morton's Mill Pond, April 3 (S:12.48), May 1 (S:0.73), June 19 (S:3.79); Calico Creek, May 2 (S:21.4); Harlowe Creek, June 22 (S:2.08).

**Modiolus demissus demissus* Dillwyn

Piver's Island, June 12; Mullet Pond, June 22, 1928; Calico Creek, July 7 (S:33.5); Core Creek Canal, July 10 (S:18.9); Eastman's Creek, July 12 (S:15.5). This is the common mussel about Beaufort.

Modiolus tulipus* LamarckLithophaga bisulcata* d'Orbigny

Bogue Sound, March 30, 1934. The boring "date shell" penetrates the shells of other molluscs.

Modiolaria lateralis* SayCongeria leucophaeta* Conrad**Polymesoda caroliniana* Bosc

Harlowe Creek, April 2 (S:0.0) June 22 (S:3.5); stream above Morton's Mill Pond, May 1 (S:0.73), July 9 (S:18.23); Core Creek Canal, July 10 (S:3.55). This clam lives in mud among the roots of water plants in swampy places along streams.

Gouldia mactracea* LinsleyVenericardia tridentata* Say**Chama congregata* Conrad**Echinochama arcinella* L.**Divaricella quadriculcata* d'Orbigny**Loripinus chrystomata* Philippi**Cardium isocarida* L.**Cardium muricatum* L.

Breakwater Flat, July 13, 1928. Fairly common along edges of channel between Beaufort and Bird Island Shoal.

Trigonocardia serrata* (L.)Laevicardium mortoni* Conrad

**Dosinia discus* Reeve

Bird Island Shoal, July 16, 1934. Common about one foot below surface of bottom; Shark Shoal, etc.

Macrocallista nimbosa Solander

Breakwater Flat, June 13, 1928.

Musculium partumieum Say

Open Grounds, ditch, June 20 (S:0.5); Calico Creek, June 21 (S:0.17); Carteret Lodge Pond, June 28 (S:0.05).

Macrocallista maculata* L.Pitar morrhuana* Linsley**Chione cancellata* L.

Piver's Island, June 12, 1928.

**Chione latilirata* Conrad

The "dog clam" is common among eel grass.

**Venus mercenaria mercenaria* L.

Piver's Island, June 12, 1928. This is the common "hardshell" clam or "quahog" about Beaufort.

**Venus campechiensis campechiensis* Gmelin

Piver's Island, June 12, 1928.

Venus ziczac* L.*Gemma gemma purpurea* H. C. LeaPtericola pholadiformis pholadiformis* Lamarek

Core Creek, June 25, 1934.

Ptericola pholadiformis lata* DallTellina lintea* Conrad**Tellina alternata* Say

On northwest end of Shark Shoal, just below low-tide mark.

**Tellina tenera* Say

Morton's Mill Pond, April 3 (S:2.48); Mullet Pond, April 30 (S:8.42); June 18 (S:12.54), June 26 (S:12.6); Core Creek Canal, July 10 (S:18.9).

**Tellina versicolor* Cozzens

**Donax fossor fossor* Say

**Donax variabilis* Say

Abundant along the beaches of Bogue Sound.

**Tagelus gibbus* Spengler

On mud flats; common. This is the "short razor" clam.

**Tagelus divisus* Spengler

A small species abundant below low-tide mark on shoals near sea.

**Ensis directus* Conrad

Breakwater Flat; June 13, 1928. This is the "razor" clam.

**Mactra fragilis* Gmelin

**Spissula solidissima solidissima* Dillwyn

Abundant off Bird Island and Shackleford Shoals.

**Spissula solidissima similis* Say

Bird Island Shoal, July 16, 1928.

**Mulinia lateralis lateralis* Say

**Rangia canaliculata* Say

Sheeps' Head Shoal, June 15, 1928.

**Mya arenaria* L.

Found in turtle ponds on Piver's Island.

**Corbula contracta* Say

**Pholas costata* L.

Sheeps' Head Shoal, June 15, 1928.

**Martesia cuneiformis* Say

Bores in oysters and other molluscs.

Bankia gouldi (Bartsch)

Piver's Island, August 6, 1928; Bird Island Shoal, August 10, 1928;
Core Creek, June 25, 1934.

Cephalopodea

Loligo pealei pealei Lesueur

Core Creek Inlet, June 16, 1928; June 25, 1934. This squid is often taken in trawls. Egg masses were common on Fort Macon beach, May 15, 1920.

Arthropoda

Crustacea

Hay and Shore (1918) published an account of the decapod crustaceans of the Beaufort region. Their list includes 153 species, many of which were collected in deep water offshore.

Crustacea

Cirripedia

Beaufort barnacles are well described in Pilsbry's (1916) monograph.

Balanus eburneus Gould

This acorn barnacle occurs chiefly below low-water mark; "often in brackish and even fresh water." During the present investigations it was often found far inland: Adam's Creek, April 2 (S:0.00); Morton's Mill Pond, April 3 (S:2.48), June 19 (S:4.9); Harlowe Creek, June 22 (S:2.08); July 2 (S:26.0); Open Ground, July 3 (S:0.07); Eastman's Creek, July 12, 1934 (S:15.52).

Balanus improvisus Darwin

Usually below low-water mark. Found in a ditch in the Open Grounds, June 18, 1928 (S:0.07) with such freshwater animals as bream, largemouth bass, water bugs, gyrrinids, and crayfishes.

Balanus amphitrite Darwin

Fort Macon Beach, June 27, 1928 (S:34.87).

Chelonibia caretta (Spengler)

From carapace of green turtle, Town Marsh, June 25, 1928; logger-head turtle, Bogue Sound, May 11, 1936.

Dichelaspis mülleri Coker

In branchial cavity of *Uca minax* (Le Conte), Harlowe Creek, April 3, 1934 (S:0.01).

Cladocera

Acantholeberis curvirostris (O. F. Müller)

Open Grounds, June 20, 1934 (S:0.4).

Alona affinis (Leydig)

Open Grounds, June 20 (S:0.4); Carteret Lodge Pond, June 28, 1934 (S:0.05).

Simocephalus serrulatus Koch

Open Grounds, June 20 (S:0.4); Carteret Lodge Pond, June 28, 1934 (S:0.05).

Copepodia

All copepods were identified by Dr. C. B. Wilson and are described in his paper (1932) on the copepods of the Woods Hole region.

Pseudocalanus elongatus Boeck

Stream above Morton's Mill Pond, May 1 (S:0.73); Calico Creek, June 21, 1934 (S:29.2).

Macrocylops albidus (Jurine)

Calico Creek, May 2 (S:0.76); Morton's Mill Pond, June 19 (S:4.9); Open Grounds, June 20 (S:0.3); Carteret Lodge Pond, June 28, 1934 (S:0.05).

Macrocylops fuscus (Jurine)

Carteret Lodge Pond, June 28 (S:0.05); Mullet Pond, July 11, 1934 (S:3.2).

Eucyclops serrulatus (Fischer)

Harlowe Creek, July 5 (S:26.0); Mullet Pond, July 11, 1934 (S:3.2).

Acartia clausii Giesbrecht

Mullet Pond, April 30 (S:8.42); Harlowe Creek, July 5 (S:26.0); Core Creek, July 12, 1934 (S:31.42).

Acartia longiremis (Lilljeborg)

Mullet Pond, April 30, 1934 (S:8.42).

Amphiascus pallidus Sars

Mullet Pond, June 18, 1934 (S:13.0).

Echinostoma normani T. & A. Scott

Mullet Pond, July 11, 1934 (S:19.8).

Ostracodia

All ostracods were identified by Dr. Norma C. Furtos.

Cypridopsis vidua vidua (O. F. Müller)

Morton's Mill Pond, May 1 (S:0.73); Calico Creek, May 1 (S:0.76);
Carteret Lodge Pond, June 28 1934 (S:0.05).

Cyprinotus putei Furtos

Mullet Pond, July 11 1934 (S:3.2).

Physocypria globula Furtos

Stream above Morton's Mill Pond, May 1 (S:0.73); Calico Creek,
May 1, 1934 (S:0.76).

Cypretta intonsa Furtos

Carteret Lodge Pond, June 28, 1934 (S:0.05).

Candona annae Mehes

Open Grounds, ditch, June 20, 1934 (S:0.3).

Cytheridae, sp.?

Stream above Morton's Mill Pond, May 1 (S:0.73); Mullet Pond,
June 26 (S:12.6), July 11 (S:3.2), stream below Morton's Mill Pond,
July 9 (S:18.23).

Amphipodida

Mr. C. R. Shoemaker (*) identified many of the amphipods.

Orchestia platensis Kroyer

Piver's Island, June 12, 1928.

Orchestia grillus (Bosc)

Morton's Mill Pond, April 3 (S:2.48), May 1 (S:8.95), June 19
(S:5.3); Mullet Pond, April 30 (S:8.42); stream above Morton's Mill
Pond, July 9 (S:21.83); Core Creek Canal, July 10, 1934 (S:18.9).

Gammarus locusta (L.)

Stream above Morton's Mill Pond, May 1, 1934 (S:0.73).

**Gammarus* sp. juv.

Calico Creek, June 21, 1934 (S:29.2).

**Eucrangonyx* sp.

Stream above Morton's Mill Pond, May 1, (S:0.73); Open Grounds, June 20 (S:0.3); Carteret Lodge Pond, June 28, 1934 (S:0.05).

**Carinogammarus mucronatus* (Say)

Harlowe Creek, April 2 (S:2.16); Morton's Mill Pond, April 3 (S:2.48), June 19 (S:5.3), Open Grounds, March 28 (S:0.00), Mullet Pond, April 30 (S:8.42), June 18 (S:0.00), June 26 (S:0.4), July 11 (S:19.8); John Godett Bay, June 19 (S:13.65).

Grubia compta (S. I. Smith)

Bogue Sound, March 30, 1934 (S:27.77).

Corophium cylindricum (Say)

Morton's Mill Pond, April 3, 1934 (S:2.48).

Caprella linearis L.

Bogue Sound, March 30, 1934 (S:27.77).

Isopodida

Mr. J. O. Moloney (*) identified certain species of isopods.

**Tanaïs* n. sp.

Mullet Pond, July 11, 1934 (S:19.8).

Leptochelia rapax Harger

Common in tubes in the mud in Mullet Pond, April 30 (S:8.42); June 18 (S:13.2); June 26 (S:12.6); July 11, 1934 (S:19.8).

Leptochelia savignyi Kroyer

Morton's Mill Pond, on submerged logs, April 3, 1934 (S:2.48).

Cyathura carinata Kroyer

Open Grounds, June 22, 1928; July 3 (S:0.07).

Cassidinidea ovalis (Say)

Morton's Mill Pond, April 3, 1934 (S:2.48).

Sphaeroma quadridentatum Say

Shackleford Bank, under stones, April 1, 1934.

Erichsonella attenuata (Harger)

Mullet Pond, among *Enteromorpha* filaments, April 30 (S:8.42), June 18 (S:12.8), July 11 (S:19.8), Morton's Mill Pond, June 19, 1934 (S:5.3).

Probopyrus pandalicola (Packard)

In branchial cavity of *Palaemonetes carolinus* Stimpson; Morton's Mill Pond, May 1 (S:8.95), June 19 (S:5.3), July 6 (S:18.23); Calico Creek, June 21 (S:0.16); Harlowe Creek, June 22, 1934 (S:3.5).

Leidya distorta (Leidy)

In branchial chamber of *Uca pugilator*, a male and a female together, Piver's Island, May 31, 1934.

Pseudione upogebiae Hay

In branchial cavity of *Upogebia affinis* (Say); Core Creek, June 14, 1928.

Asellus communis Say

Open Grounds, March 28, 1934 (S:0.0).

Decapodida

Quotations are from Hay and Shore (1918).

Natantina

Penaeus brasiliensis Latreille

Abundant in brackish creeks. Morton's Mill Pond, June 19 (S:5.3); Calico Creek, June 21 (S:0.16), July 6 (S:32.4); Core Creek Canal, July 10 (S:3.55); Harlowe Creek, June 22, 1934 (S:3.5).

Penaeus setiferus (L.)

Common in summer. Harlowe Creek, July 5, 26.0; Core Creek Canal, July 10, 1934 (S:3.55).

Palaemonetes carolinus Stimpson

"Abundant among eel grass and about the margins of marshes." Harlowe Creek, April 2 (S:2.16), June 22 (S:3.5); Morton's Mill Pond, April 3 (S:2.48), May 1 (S:8.95), June 19 (S:5.3), above Morton's Mill Pond, May 1 (S:0.73), July 9 (S:18.23), with eggs; Mullet Pond, April 30 (S:9.11), June 18 (S:12.54) June 26 (S:12.5), July 11 (S:0.3); Calico Creek, May 2 (S:0.76-21.4), June 21 (S:0.16); Core Creek Canal, July 10 (S:3.55); Eastman's Creek, July 12, 1934 (S:15.52).

Palaemonetes vulgaris (Say)

Often associates with the last species. Calico Creek, July 6, 1934 (S:32.0).

Palaemonetes exilipes Stimpson

Mullet Pond, June 18, young (S:1.69); Carteret Lodge Pond, June 28, 1934 (S:0.05).

Reptantina

Cambarus blandingii (Harlan)

Open Grounds, ditch, June 18, 1928 (S:0.3); July 3 (S:0.07).

Upogebia affinis (Say)

Core Creek Canal, June 14, 1928.

Emerita talpoidea (Say)

Sheep's Head Shoal, Shackleford Bank, June, 1928, 1934. Common on sandy beaches in certain localities.

Pagurus pollicaris Say

Bogue Sound, March 30, 1934, with eggs (S:27.77).

Hepatus epheliticus (L.)

Beaufort Breakwater, June 20, 1928.

Ovalipes ocellatus ocellatus (Herbst)

Sheep's Head Shoal, June 15, 1928.

Callinectes sapidus Rathbun

Open Grounds, ditch, June 22, 1928, July 3, 1934 (S:0.07); Bogue Sound, March 30 (S:27.77); Mullet Pond, April 1 (S:8.3), June 18 (S:0.05), June 26 (S:12.6), July 11 (S:32); Harlowe Creek, April 2 (S:2.16); Morton's Mill Pond, April 3 (S:2.48), June 19 (S:5.3), July 5 (S:20.4); Creek above Morton's Mill Pond, July 9 (S:21.8); Calico Creek, June 21 (S:0.16); July 6 (S:34.1); Lenoxville Road, ditch, June 30 (S:28.6); Core Creek Canal, July 10 (S:0.7-18.9); Eastman's Creek, July 12, 1934 (S:15.5).

Rithropanopeus harrisi (Gould)

Open Grounds, ditch, June 28, 1928 (S:0.05); Morton's Mill Pond, April 3 (S:2.48), June 19 (S:4.02); Harlowe Creek, June 22, 1934 (S:3.5).

Eurypanopeus depressus (Smith)

Beaufort Breakwater, June 25, 1928.

Neopanope texana sayi (Smith)

Piver's Island, June 24, 1928.

Panopeus sayi Smith

Core Creek, June 14; Piver's Island, pier, June 16, 1928.

Menippe mercenaria De Haan

Town Marsh, June 18, 1928; Bogue Sound, March 30, 1934 (S:27.77).

Pinnotheres ostreum Say

Piver's Island, June 15, 1928; in oysters.

Pinnotheres maculatus Say

Bogue Sound, March 30, 1934 (S:27.77).

Dissodactylus mellitae (Rathbun)

On sand dollars, Mellita; Bird Island Shoal; June 25, 1934.

Pinnixa sayana Stimpson

Bird Island Shoal, August 15, 1928.

Sesarma cinerea Say

Calico Creek, May 2 (S:16.52); Harlowe Creek, June 22 (S:3.3-0.5); Mullet Pond, July 11 (S:0.3); Core Creek Canal, July 10, 1934 (S:18.9).

Ocypode albicans Bosc

Common on sandy beaches above high-tide mark, where it lives in burrows.

Uca minax (Le Conte)

Open Grounds, June 18; Sheep's Head Shoal, June 15, 1928; Harlowe Creek, April 2 (S:2.2-9.3), June 22 (0.5-3.3), July 5 (S:26.0); Morton's Mill Pond, April 3 (S:2.48); Calico Creek, May 2 (S:18.5), June 21 (S:0.16), July 6 (S:33.2); above Morton's Mill Pond, July 9 (S:12.64); Lenoxville Road, June 30 (S:28.44); Core Creek Canal, July 10 (S:0.07); Eastman's Creek, July 12, 1934 (S:15.52).

Uca pugnax (Smith)

Fort Monroe, marshes, June 27, 1928.

Uca pugilator (Bosc)

Core Creek Canal, June 14, 1928; Piver's Island, March 31; Mullet Pond, April 30 (S:8.42), July 11, 1934 (S:19.8).

Insectea

Collembolida

Identified by J. W. Folsom (*)

**Anurida maritima* Guérin

Shackleford Bank, under stones, June 16, 1934.

**Bourletiella spinata* Mac. G.

On water along stream above Morton's Mill Pond, May 1, 1934 (S:0.73).

**Isotomurus palustris* Müller

On water along stream above Morton's Mill Pond, May 1, 1934 (S:0.73).

Ephemera

Identified by Dr. Jay R. Traver (*)

**Callibaetis* sp.

Carteret Lodge Pond, June 28, 1934 (S:0.05).

**Caenis diminuta* Walker

Open Grounds, ditch, June 20 (S:0.03); Carteret Lodge Pond, June 28, 1934, (S:0.05).

Odonatida

Identified by P. P. Calvert (*)

**Enallagma* sp., juv.

Mullet Pond, June 18 (S:0.05), June 26 (S:12.6), July 11 (S:3.2, 19.8); Carteret Lodge Pond, June 28, 1934 (S:0.05).

**Ischnura posita* (Hagen)

Open Grounds, ditch, June 20 (S:0.5), July 2 (S:0.05); stream above Morton's Mill Pond, May 1 (S:0.73), July 9, 1934 (S:15.5).

**Ischnura ramburii* Selys

Mullet Pond, April 30 (S:9.11), April 28 (S:8.42), June 18, June 26 (S:0.4), July 11 (S:0.3); Morton's Mill Pond, June 19 (S:5.3), July 9 (S:15.5); Open Grounds, ditch, June 20 (S:0.3, 0.5); Calico Creek, June 21 (S:0.16); Core Creek Canal, July 11, 1934.

**Ischnura verticalis* (Hagen)

Open Grounds, June 20, 1934 (S:0.3).

**Agrion maculatum* Beauvois

Open Grounds, stream, June 21, 1928, July 3, 1934.

**Pachydiplax longipennis* Burmeister

Mullet Pond, June 22, 1928; Open Grounds, ditch, June 20; Core Creek Canal, July 10, 1934.

**Anomalagrion hastatum* (Say)

Open Grounds, June 20; Calico Creek, June 21; Mullet Pond, June 28; stream above Morton's Mill Pond, July 9, 1934.

**Argia fumipennis* (Burmeister)

Open Grounds, ditch, June 20, July 3, 1934.

**Calopteryx maculata* (Beauvois)

Open Grounds, stream, July 3, 1934.

**Anthax sinuosa* Wied.

Morton's Mill Pond, June 19, 1934.

**Erythrodiplox berenice berenice* (Drury)

Mullet Pond, June 18, June 26, July 11; Morton's Mill Pond, June 19; Lenoxville Road, June 30; Open Grounds, ditch, July 2, July 3, 1934.

**Somatochlora linearis* (Hagen)

Eastman's Creek, July 12, 1934.

**Libellula auripennis* Burmeister

Morton's Mill Pond, June 19; Calico Creek, June 21; Carteret Lodge Pond, June 28; stream above Morton's Mill Pond, July 9, 1934.

**Libellula vibrans* Fabricius

Open Grounds, stream; July 3, 1934.

**Libellula incesta* Hagen

Carteret Lodge Pond, June 28, 1934.

**Libellula semifasciata* Burmeister

Open Grounds, June 20; Carteret Lodge Pond, June 28, 1934.

**Celithemis eponina* Drury

Carteret Lodge Pond, June 28, 1934.

**Erythemis simplicicollis* (Say)

Mullet Pond, April 30, June 18; Carteret Lodge Pond, June 28, 1934.

**Pachydiplax longipennis* Burmeister

Mullet Pond, April 30, June 26; Open Grounds, June 20; Carteret Lodge Pond, June 28; Core Creek Canal, July 10, 1934.

**Plathemis lydia* (Drury)

Calico Creek, June 21, 1934.

**Anax junius* (Drury)

Mullet Pond, July 11, 1934, nymph (S:0.3).

**Gomphaeschna furcillata* (Say) *antelope* Hagen

Open Grounds, June 20; stream above Morton's Mill Pond, July 9, 1934.

**Coryphaeschna ingens* (Rambur)*Heteropterida*

Bugs were identified by H. B. Hungerford (*) and C. S. Brimley (**).

**Saldula reperta* Uhlmán

Mullet Pond, July 11, 1934.

***Gerris nebularis* Drake & Hottes

Open Grounds, June 28, 1928.

**Gerris* sp., juv.

Stream above Morton's Mill Pond, May 1; Open Grounds, June 20, July 3; Carteret Lodge Pond, June 28, 1934.

***Arctocorixa* sp.

Mullet Pond, June 22, June 28, 1928, April 30, 1934.

**Hydrometra martini* Kirk

Open Grounds, ditch, June 20; Carteret Lodge Pond, June 28, 1934.

**Rheumatobates tenuipes* Meinert

Stream above Morton's Mill Pond, July 9, 1934.

Benacus griseus Say

Open Grounds, June 18; 1928**; Carteret Lodge Pond, June 28, 1934.

Belostoma sp., juv.

Mullet Pond, June 18, 26, July 11; Open Grounds, ditch, June 20, 1934.

Notonecta irrorata Say

Open Grounds, June 18, 1928**, June 6, 1934*; Harlowe Creek, June 22, 1934*.

**Notonecta* sp., juv.

Mullet Pond, June 18, 1934.

**Mesovelia mulsanti* White

Carteret Lodge Pond, June 28; Mullet Pond, July 11, 1934.

**Trichocorixa verticalis* Fieber

Stream above Morton's Mill Pond, May 1; Mullet Pond, June 18, July 11; Open Grounds, creek, July 2, 1934.

**Trichocorixa* sp., juv.

Mullet Pond, July 11, 1934.

*Homopterida**Tibicen chloromera* (Walker)

Near Harlowe Creek, June 22; near Eastman's Creek, July 12, 1934.

Orthopterida

Orthopterans were identified by A. N. Caudell (*).

**Hippiscus phoenicopterus* (Burmeister)

Open Grounds, June 20, 1934.

**Homocoryphus malivolans* (Scudder)

A specimen of this species was captured on Piver's Island during the summer of 1934.

**Leptysma marginicollis* (Serville)

Mullet Pond, swamp, June 26, 1934.

**Limnogonus* sp.

Carteret Lodge Pond, June 28, 1934.

**Manomera tenuescens* (Scudder)

Piver's Island, summer of 1934.

**Mermiria* sp.

Lenoxville Road, June 30; Open Grounds, July 2, 3, 1934.

**Oncometopia undata* Fabricius

Harlowe Creek, June 22, 1934.

**Orchelium fidicinium* Rehn & Hebard

Calico Creek, June 21; Lenoxville Road, June 30; Eastman's Creek, July 12, 1934.

**Paroxya clavuliger* (Serville)

Mullet Pond, June 26, Open Grounds, July 2, 3, 1934.

**Pissonotus* sp.

Carteret Lodge Pond, June 28, 1934.

Coleopterida

Beetles were identified by H. S. Barber, A. G. Bovig, C. S. Brimley, L. L. Buchanan, W. S. Fisher, and P. W. Oman.

Berosus infuscatus Lec.

Mullet Pond, July 11, 1934.

Berosus peregrinus Herbst

Carteret Lodge Pond, June 28, 1934.

Canthydrus bicolor Say

Carteret Lodge Pond, June 28, 1934.

Centrinaspis sp.

Morton's Mill Pond, June 19, 1934.

Chauliognathus marginatus (Fabricius)

Morton's Mill Pond, June 19, 1934.

Chrysobothris sp.

Mullet Pond, June 18, 1934.

Cicindela hirticollis Say

Mullet Pond, June 18, 1934.

Cicindela lacerta Chd.

Mullet Pond, June 18, 1934.

Cicindela tortuosa Lec.

Mullet Pond, June 18, July 11, 1934.

Copelatus glyphicus Say

Open Grounds, ditch; June 20, 1934.

Dineutes carolinus Lec.

Open Grounds, stream, July 3; Morton's Mill Pond, July 9, 1934.

Dineutes emarginatus Say

Carteret Lodge Pond, June 20; stream above Morton's Mill Pond, July 9, 1934.

Dineutes serrulatus Leconte

Open Grounds, June 18, 1934.

Enochrus sublongus Fall

Open Grounds, March 28, June 20; Mullet Pond, April 30, 1934.

Enochrus perplexus Lec.

Mullet Pond, April 4, June 18, 26, July 11, 1934.

Galerucella nymphaeae (L.)

Carteret Lodge Pond, June 28, 1934.

Gyrinus analis Say

Open Grounds, ditch, June 18, 1928, June 20, July 2, 1934.

Hydaticus bimarginatus Say

Mullet Pond Swamp, April 30; Eastman's Creek, July 14, 1934.

Hydroporus carolinus Fall

Open Grounds, ditch, June 20, 1934.

Hydroporus clypeatus Sharp

Open Grounds, ditch, June 20; Harlowe Creek, June 22, 1934.

Lixus marginatus Say

Open Grounds, ditch, June 20, 1934.

Megamelus sp.

Carteret Lodge Pond; June 28, 1934.

Melanactes piceus (De G.)

Harlowe Creek, June 22, 1934.

Monochamus titillator Fabricius

Eastman's Creek; July 12, 1934.

Ranthus calidus Fabricius

Open Grounds, June 28, 1934.

Tropisternus glaber Herbst

Open Grounds, June 28, 1928; Mullet Pond, July 11, 1934 (larva).

Topisternus quadristriatus Horn

Open Grounds, pond, March 28; Mullet Pond, March 28, April 1, 30, July 11, 1934.

Dipterida

Dipterous insects were identified by C. S. Brimley, C. T. Greene, G. A. Sandhouse, A. Stone, and the writer.

Aedes canadensis (Theobald)

Core Creek Canal, July 10, 1934.

Aedes sollicitans (Walker)

Open Grounds, ditches and streams, June 20, July 3, 1934.

Anopheles crucians Wiedemann

Open Grounds, June 20, 1934.

Anthrax sinuosa Wiedemann

Morton's Mill Pond; June 19, 1934.

Cerceris fumipennis Say

Morton's Mill Pond, June 19, 1934.

Chaetopsis aenea Wiedemann

Calico Creek, June 21; Eastman's Creek, July 12, 1934.

Chaetopsis fulvifrons Macq.

Stream above Morton's Mill Pond, May 1, June 19. Morton's Mill Pond, June 19; Calico Creek, June 21, 1934.

Chironomus lobiferus Say

Above Morton's Mill Pond, May 1; Calico Creek, May 2; Open Grounds, ditch and stream, June 20, July 3, 1934.

Chironomus flavus Johannsen

Carteret Lodge Pond, June 28, 1934.

Chironomus sp.

Mullet Pond, April 30, June 18, July 11, 1934.

Chrysops vittata floridana Johnson

Harlowe Creek, June 22, 1934.

Diachlorus ferrugineatus Fabricius

Open Grounds, June 20, July 2, 3; Harlowe Creek, June 22; Carteret Lodge Pond, June 28; Morton's Mill Pond, July 9, 1934.

Dolichopodidae

Harlowe Creek, June 22, 1934.

Empidae

Mullet Pond, April 30, 1934.

Ephydra sp.

Mullet Pond, April 30, 1934.

Erax albibarbis Macq.

Mullet Pond, June 18, 1934.

Hoplodictya spinicornis Lw.

Mullet Pond, April 30, 1934.

Hydrophorus sp.

Open Grounds, March 29; Mullet Pond, April 1, June 18, July 11 (pupal cases), 1934.

Laphystia litoralis Curran

Mullet Pond, June 18, 1934.

Lauzania facialis Coq.

Mullet Pond, April 30, 1934.

Mesogramma polita Say

Calico Creek, June 21, 1934.

Mydas clavatus Drury

Harlowe Creek, June 22, 1934.

Pelastoneurus parvus Aldrich

Mullet Pond, April 30, 1934.

Psorophora ciliata (Fabricius)

Core Creek Canal, July 10, 1934.

Psychodidae

Morton's Mill Pond, June 19, 1934—pupae.

Pteticus sakeni Williston

Open Grounds, June 28, 1928.

Saldula reperta Uhl.

Mullet Pond, July 11, 1934.

Sarcophaga sarracenioidea Aldrich

Open Grounds, June 18, 1928.

Stratiomyidae

Mullet Pond, April 30, 1934; larvae among algae along shore.

Tabanus costalis Wiedemann

Open Grounds, June 28, 1928.

Tabanus lineola Fabricius

Open Grounds, June 28, 1928.

Tanypus decoloratus Malloch

Open Grounds, June 20; Carteret Lodge Pond, June 28; Mullet Pond, July 10, 1934.

Tipulidae

Along the shores of Mullet Pond on April 30, 1934, crane-fly larvae were common in the sand along shore, and adults in the air. Through an unfortunate accident none was saved for identification.

Merostomata

Xiphosura

Limulus polyphemus (L.)

Beaufort Breakwater, June 13, 1928; Bogue Sound, March 30, Town Marsh Flats, June 27, Mullet Pond, July 11, 1934.

Arachnida

Acarina

Mites were identified by Dr. Ruth Marshall.

Arrhenurus marshallae Piers

Morton's Mill Pond, May 1, 1934 (S:2.0).

Arrhenurus megalurus Marshall

Morton's Mill Pond; June 28, 1934 (S:0.05).

Eylais desecta Koenike

Mullet Pond, swamp; April 1, 30, 1934.

Koenikea concava Wol.

Carteret Lodge Pond, June 28, 1934 (S:0.05).

Koenikea haldemanii Viets

Morton's Mill Pond, June 28, 1934 (S:0.05).

Oribatidae

Morton's Mill Pond, June 28, 1934 (S:0.05).

Araneida

Spiders were identified by Dr. A. M. Chickering.

Dolomedes sp., juv.

Stream above Morton's Mill Pond; Open Grounds, June 20, 1934.

Pardosa pauxilla Montgomery

Mullet Pond, April 30, 1934.

Tetragnatha pallescens Cambridge

Mullet Pond, April 30; above Morton's Mill Pond, May 1, 1934.

Tetragnatha laboriosa Hentz

Swamp above Morton's Mill Pond, May 1, 1934.

Enteropneusta

Balanoglossus aurantiacus (Girard)

Common on the flats near Beaufort; Beaufort Breakwater, July 5, 1928.

Chordata

Piscea

Fishes about which the writer was in doubt were referred to S. F. Hildebrand.

Anguillula rostrata (Lesueur)

Open Grounds, ditch, and stream, June 28, 1928; July 2 (S:0.07); stream above Morton's Mill Pond, May 1 (S:0.73); Harlowe Creek, June 22 (S:0.5); Calico Creek, May 2 (S:0.76), June 21 (S:0.16); Lenoxville Road, June 30, 1934 (S:28.5).

Aphredoderus sayanus (Gilliams)

Open Grounds, ditch; June 28, 1928; June 20 (S:0.5), July 3, 1934 (S:0.07).

Chasmodes bosquianus (Lacépède)

Core Creek, June 14, 1928.

Ctenogobius stigmaticus (Poey)

Harlowe Creek, April 2, 1934 (S:9.27).

Cynoscion regalis (Block & Schneider)

Core Creek Canal, July 10, 1934 (S:22.5).

Cyprinodon variegatus Lacépède

Mullet Pond, June 28, 1928 (S:20.4), June 18. (S:0.05), June 26 (S:12.6), July 11 (S:19.8); Eastman's Creek, July 12, 1934 (S:15.5).

Elassoma zonatum Jordan

Open Grounds, ditch; June 20, 1934 (S:0.3).

Enneacanthus gloriosus (Holbrook)

Carteret Lodge Pond, June 28, 1934 (S:0.05).

Esox americanus (Gmelin)

Open Grounds, ditch, June 18, 1928 (S:0.03).

Eupomotis gibbosus (L.)

Open Grounds, June 18, 1928 (S:0.03); Morton's Mill Pond, April 3, 1934 (S:2.48).

Fundulus diaphanus (Lesueur)

Calico Creek, May 2 (S:0.76), June 21 (S:0.16); Morton's Mill Pond, June 19 (S:5.7); Harlowe Creek, June 22, 1934 (S:0.5).

Fundulus heteroclitus (L.)

Core Creek Inlet, June 14; Fort Macon, marsh, June 27, 1928 (S:34.8); Lenoxville Road, ditch, June 29, 1934 (S:21.4).

Fundulus majalis (Wahlbaum)

Harlowe Creek, April 2 (S:2.2); Calico Creek, July 6 (S:32.0); Core Creek Canal, July 10 (S:21.5); Eastman's Creek, July 12 (S:15.5).

Gambusia holbrooki Girard

Open Grounds, ditch, June 18, 1928, June 20 (S:0.5), July 2, 1934 (S:0.05); Mullet Pond, June 22, 1928 (S:20.4), June 18 (S:0.03), July 11, 1934 (S:0.3-3.2); Morton's Mill Pond, April 3 (S:2.48), May 1 (S:8.95), June 19 (S:5.3); Harlowe Creek, April 2 (S:2.16), May 1 (S:0.05); above Morton's Mill Pond, May 1 (S:0.73), July 9; Carteret Lodge Pond, June 28 (S:0.05); Lenoxville Road, ditch, June 30 (33.0); Core Creek Canal, July 10 (S:0.7); Calico Creek, July 6 (S:32.7).

Gobiosoma boscii Lacépède

Mullet Pond, June 26, 1934 (S:12.6).

Hypsoblennius hentz (Lesueur)

Beaufort Breakwater, June 13 (S:32.0); Piver's Island, June 15, 1928 (S:31.3).

Leiostomus xanthurus Lacépède

Common near Beaufort; Core Creek Canal, July 10, 1934 (S:18.9).

Menidia menidia (L.)

Core Creek Canal, July 10, 1934 (S:18.9).

Menidia bryllina (Cope)

Open Grounds, ditch, July 18, 1928 (S:0.5); Mullet Pond, June 18 (S:0.05), June 22 (S:20.2-26.9), June 26 (S:12.6), July 11, 1934 (S:19.8).

Menticirrhus saxatilis (Bloch & Schneider)

Mullet Pond, June 26, 1934 (S:12.6).

Micropogon undulatus (L.)

The croaker is common in the Beaufort region. Open Grounds, June 18, 1928 (S:0.5); Morton's Mill Pond, April 3 (S:2.48), May 1 (S:8.95); stream above Morton's Mill Pond, May 1 (S:0.73); Core Creek Canal, July 10 (S:25.8).

Micropterus salmoides (Lacépède)

Open Grounds, ditch, June 18, 1928.

Mugil cephalus L.

Core Creek, July 10, 1934 (S:25.8).

Mugil curema Cuvier & Valenciennes

Morton's Mill Pond, April 3 (S:2.48); Calico Creek, July 6, 1934 (S:34.1).

Notemigonus chrysoleucas (Mitchill)

Open Grounds, ditch, June 18, 1928.

Notropis hudsonius (Clinton)

Open Grounds, ditch, June 18, 1928.

Opsanus tau (L.)

The toadfish is common near Piver's Island and elsewhere in the Beaufort region.

Orthopristis chrysopterus (L.)

Core Creek, June 25; Mullet Pond, June 26 (S:12.6); Core Creek Canal, July 10, 1934 (S:25.8).

Paralichthys dentatus (L.)

Open Grounds, July 18, 1928; Morton's Mill Pond, June 19, 1934 (S:5.3).

Pteroplatea maculura (Schneider)

Core Creek Inlet, June 16, 1928.

Spheroides maculatus Block & Schneider

Beaufort Breakwater, June 25, 1928.

Symphurus plagiusa (L.)

Core Creek Canal, June 25, July 10, 1934.

Amphibia

Frogs and toads were identified by B. B. Brandt.

Acris gryllus (Le Conte)

Open Grounds, March 29; Carteret Lodge Pond, June 28, 1934.

Bufo fowleri Garman

Open Grounds, June 20, 1934.

Hyla squirella Latreille

Mullet Pond, swamp, June 26 (S:0.4).

Rana catesbeiana Shaw

Carteret Lodge Pond, June 28, 1934.

Rana sphenoccephala (Cope)

Mullet Pond, June 18 (S:0.3), June 26 (S:0.4); Morton's Mill Pond (S:4.9); Harlowe Creek, June 22 (S:3.5-0.5); Open Grounds, March 29 (S:0.3), July 2 (S:0.0), July 3 (0.07); Calico Creek, June 21 (S:0.16); Carteret Lodge Pond, June 28 (S:0.05); Lenoxville Road, ditch, June 30 (S:21.4); Core Creek Canal, July 10, 1934 (S:0.7).

Reptilea

Alligator mississippiensis (Daudin)

Carteret Lodge Pond, June 28, 1934 (S:0.05).

Anolis carolinensis Cuvier

Near Mullet Pond, July 21, 1928, June 18, Carteret Lodge Pond, June 28, 1934.

Caretta caretta (L.)

Loggerhead turtles are often taken by fishermen in the sounds near Beaufort.

Chelone mydas (L.)

Green turtles are often captured in the sounds near Beaufort.

Chelopus guttatus (Schneider)

Open Grounds, June 20, 1934 (S:0.0).

Chelydra serpentina (L.)

Mullet Pond, Swamp, July 11, 1934 (S:0.3).

Kinosternon subrubrum (Lacépède)

Open Grounds, March 28, June 21, July 3; Mullet Pond, Swamp, June 18; Harlowe Creek, June 22, 1934.

Avea

Many birds were observed, but no record was kept of their occurrence. Coues and Yarrow (1878) give a list of the birds they observed in the Beaufort region.

Mammalea

Prodelphinus plagiodon (Cope)

Porpoises ranged through the sounds near Beaufort, but were never seen in the streams.

LIST OF PLANTS OBSERVED

Thallophyta

Hoyt (1921) has published a list of the algae of the Beaufort region.

Enteromorpha intestinalis (L.) Grev.

Mullet Pond, April 30 (S:9.11); Calico Creek, May 2, 1934 (S:0.76).

Microspora sp.

Open Grounds, pond; March 28, 1934 (S:0.03).

Ulva lactula L.

Adams Creek, April 2, 1934 (S:0.03).

Bryophyta

Sphagnum sp.

Open Grounds, pond, March 28 (S:0.03), June 20 (S:0.3).

Spermatophyta

Juncus raemerianus Scheele

This sedge was common on swampy areas along brackish inlets. Above Morton's Mill Pond, May 1 (S:0.73), July 9, 1934 (S:12.64-18.23).

Sabal glabra (Mill.) Sarg.

Dwarf palmettos often occur along brackish water areas near Beaufort. Open Grounds, stream, July 3 (S:0.07); Core Creek Canal, July 6, 1934 (S:18.9).

Salicornia perennis Mill

Eastman Creek, July 12, 1934; common on flats (S:27.7).

Typha angustifolia L.

Open Grounds, ditch, March 20 (S:0.00); Mullet Pond, Swamp, June 18 (S:0.03); along stream above Morton's Mill Pond, July 9, 1934 (S:18.2).

TOLERATION OF MARINE AND ESTUARINE ANIMALS TO VARIATIONS
IN SALINITY

During the summer of 1928 Miss Sophie Dehler carried on a series of experiments for the writer to determine the ability of littoral marine

TABLE 1

Average time in hours that various animals lived in fresh water, sea water, and in various mixtures of the two

+ after a number indicates that animals were alive and in good condition when an experiment was discontinued.

ANIMAL	NUM- BER USED	FRESH	1:3	1:1	3:1	SEA WATER
Annelid:						
<i>Chaetopterus pergamentaceus</i> Cuv.....	20	10.5	10.5	16.5	28.5	28.5
Ophiuroid:						
<i>Ophiura brevispina</i> Say.....	35	16	20.5	35	83+	83+
Echinoids:						
<i>Arabacia punctulata</i> (Lamarck).....	10	14.5	14.5	22	64	51
<i>Lytechinus variegatus</i> (Lamarck).....	30	16	16	16.5	35	71
<i>Mellita quinquesperforata</i> (Clark)....	20	8	26	56.5	68.5	436+
Snails:						
<i>Busycon p. perversum</i> (L.).....	10	17	42	60	606+	1095+
<i>Crepidula fornicata</i> (Verrill).....	10	20.5	20.5	33	33	33
<i>Fasciolaria tulipa</i> L.....	25	8	8	14	30	31
<i>Nassarius obsoleta</i> Say.....	15	42	42	1124+	1124+	1124+
<i>Oliva s. sayana</i> Ravenel.....	18	15	32.5	121+	199+	221+
<i>Polynices duplicata</i> Say.....	24	63	69	140+	194+	264+
<i>Strombus p. pugilis</i> L.....	9	16	24	119	214+	253+
<i>Terebra dislocata</i> Say.....	40	15.5	19	476+	595+	639+
<i>Urosalpinx cinereus</i> (Say).....	30	20	28	796+	796+	796+
Clams:						
<i>Bankia gouldi</i> Bartsch.....	14	15	106+	106+	117+	23
<i>Cardium muricatum</i> L.....	10	15	15	15	746	984+
<i>Chione cancellata</i> L.....	20	85	102	157	174	736+
<i>Dosinia discus</i> Reeve.....	10	54	102	308	284	114
<i>Lucina filosa</i> Stimpson.....	10	27	125	196+	281+	281+
<i>Spissula solidissima similis</i> Say.....	12	15	32	48	96	159
<i>Modiolus d. demissus</i> Dillwyn.....	10	148	330+	330+	330+	330+
<i>Ostrea virginica</i> Gmelin.....	13	70	70.5	288+	271+	382+
<i>Pecten i. irradians</i> Lamarck.....	25	8	8	14	30	31
<i>Sinum perspectivum</i> Say.....	20	24.5	33	106+	151	110
<i>Solemya velum</i> Say.....	80	20	22	32	218	190
<i>Tagelus gibbus</i> Spengler.....	20	16	37	40	53	41
<i>Tellina alternata</i> Say.....	4	15	21	21	21	—
<i>Venus m. mercenaria</i> L.....	20	114+	102+	120+	120+	120+
Barnacle:						
<i>Balanus eburneus</i> Gould.....	12	24.5	63	164	305	305
Amphipod:						
<i>Orchestia platensis</i> Kroyer.....	22	1	14	270	172	16

TABLE 1—*Concluded*

ANIMAL	NUM- BER USED	FRESH	1:3	1:1	3:1	SEA WATER
Hermit, Crabs, etc.:						
<i>Clibanarius vittatus</i> (Bosc).....	10	20	432+	432+	432+	432+
<i>Emerita talpoidea</i> (Say).....	20	26	11	21	206	14.5
<i>Eurypanopeus depressus</i> (Smith).....	10	27.5	904+	892+	910+	910+
<i>Pagurus longicarpus</i> Say.....	10	20	240+	432+	432+	432+
<i>Panopeus herbstii</i> H. Milne-Edw.....	10	21.5	504+	504+	504+	504+
<i>Pinnixa chaetoptera</i> Stimpson.....	10	4.5	7	43	408	408
<i>Polyonyx macrocheles</i> (Gibbes).....	71	11	11	11	138+	214+
					96	165+
<i>Sesarma cinerea</i> Say.....	10	87	289	780+	713+	181
<i>Uca pugnax</i> (Smith).....	25	28	1213+	1059+	1114+	1052+
Xiphosuran:						
<i>Limulus polyphemus</i> (L.).....	20	38	279+	714+	775+	714+
Tunicate:						
<i>Molgula manhattensis</i> (De Kay).....	10	14	23	23	31	62
Fishes:						
<i>Fundulus heteroclitus</i> (L.).....	10	598+	317	401	408	271
<i>Fundulus majalis</i> (Lesueur).....	17	12	252	267	283	284
<i>Lagodon rhomboides</i> (L.).....	5	1	1.5	1	16.5	16.5
<i>Menidia bryllina</i> (Cope).....	5	0.5	1	64	16	40

and estuarine animals to live in dilutions of sea water. The work was done between June 10 and August 31. Temperatures in the dishes where marine animals were kept in the laboratory varied between 22.3°C. and 29.6°C. Salinities of the running sea water supplied in the laboratory varied between 32‰ and 38‰ (Gutsell 1931). The results of Miss Dehler's tests are shown in Table 1. Perhaps the deaths of some of the animals were due to the conditions of the experiments, but it is perhaps significant that none lived longer in fresh water than in dilutions of sea water. Of the forty-five specimens tested only eight lived longer in brackish water than in undiluted sea water. These were (1) *Bankia*, *Modiolus*, and *Ostrea* (which are typical brackish water animals) (2) *Orchestia* and *Sesarma* (which are terrestrial animals that live near the sea), *Fundulus heteroclitus* (a fish which migrates freely from salt to fresh water) and *Emerita* (a burrowing crustacean which lives on sandy bottoms where there are strong currents and waves).

Among the animals that were found in localities, such as Mullet Pond, Harlowe Creek, Calico Creek, and Morton's Mill Pond, where

salinities were continually fluctuating and often low, the following may be mentioned:

Morton's Mill Pond (S:2.48-20.6): Marine types, such as blue crabs, *Palaemonetes carolinus*, *Peneaus brasiliensis*, Tellina, Corophium, Leptochelia, Orchestia, *Nereis limbata*, Rhythropanopeus, Elysia, mussels, *Balanus eburneus*, *Uca minax*, *Gammarus locusta*, Mugil, Micropogon, Nemopsis, flounder, and Dactylometra, were associated with fresh water types of chironomid larvae, Gambusia, Carinogammarus, Polymesoda, *Fundulus diaphanus*, and *Macrocyclus albidus*.

Calico Creek (S:0.16-20.0): with Paludestrina, Littoridina, *Littorina irrorata*, *Nereis limbata*, Sesarma, *Uca minax*, *Callinectes sapidus*, *Palaemonetes carolinus*, *Peneaus brasiliensis*, and *Mytilus exustus* were found Gambusia, *Fundulus diaphanus*, eel, several odonates, *Chironomus lobiferus*, and *Microcyclus albidus*.

Harlowe Creek (S:0.00-26.0): with *Uca minax*, Sesarma, *Callinectes sapidus*, *Peneaus brasiliensis* and *P. setiferus*, *Palaemonetes carolinus*, *Balanus eburneus*, and *Ostrea virginica* lived Polymesoda, Gambusia, chironomid larvae, bugs, flies, leopard frog, and eels.

Mullet Pond (S:8.3-13.1) with Enteromorpha, *Callinectes*, *Uca pugilator*, Orchestia, *Palaemonetes carolinus*, Erichsonella, Leptochelia, Tellina, Littoridina, *Crepidula fornicata*, *Leptonereis culveri*, *Glycera americana*, Myxobdella, Edwardsia, Rotalia, Menidia, Cyprinodon, Menticirrhus, Orthopristia, lived a number of species of beetles, dragonflies, damselflies, bugs, and dipteridans.

Open Grounds (0.0-03): the blue crab, Rhythropanopeus, *Uca minax*, *Balanus improvisus*, *B. eburneus*, *Peneaus brasiliensis*, *Palaemonetes carolinus*, and *Menidia bryllana* were associated with crayfishes, Gambusia, Esox, Eupomotis, Aphredoderus, Anguilla, Notemigonus, Natrix, Rana (and tadpoles), Kinosternon, Succinea, and a variety of insects (beetles; damsel-, dragon-, and may-flies; and bugs).

Arthropods and fishes are the types that best tolerate extreme variations in salinity. When sea water becomes extremely dilute a few marine types of crustaceans and fishes persist; when the sea water is diluted only slightly with fresh water representatives of comparatively specialized orders (Coleoptera, Diptera) and of some more primitive orders (Odonata, Heteroptera) are the first to enter it.

TOLERATION OF DESICCATION BY ESTUARINE ANIMALS

During the summer of 1928 Miss Dehler tested the ability of certain estuarine animals to endure desiccation. Animals were placed in open,

TABLE 2

Length of time in hours that certain estuarine animals live when exposed to desiccation in air; June, July 1928

SPECIES	NUMBER TESTED	AVERAGE	MAXIMUM
Anemone:			
<i>Sagartia luciae</i> (Verrill) ..	10	25 8	28 0
Echinoderms:			
<i>Ophiura brevispina</i> (Say) ..	11	3 1	4 3
<i>Arbacia punctulata</i> (Lamarck)	10	47 0	47 0
Snails:			
<i>Fasciolaria tulipa</i> L.....	10	72 8	91 1
<i>Littorina irrorata</i> Say. . .	10	184+	184+
<i>Nassarius obsoleta</i> Say ..	10	58 3	64 8
<i>Urosalpinx cinereus</i> (Say).	10	44 3	57 5
Clams:			
<i>Chione cancellata</i> L. . .	10	49 2	66 0
<i>Modiolus d. demissus</i> Dillwyn	10	209+	209+
<i>Ostrea virginica</i> Gmelin	31	115 1	210.1
<i>Pecten i. irradians</i> Lamarck	11	18 8	18 9
<i>Solemya velum</i> Say.	10	19 0	19 0
<i>Venus mercenaria</i> L..	10	255 8	310.1
Barnacle:			
<i>Balanus eburneus</i> Gould	10	82 5	93 0
Hermit crabs:			
<i>Clibanarius vittatus</i> (Bosc)	10	141 0	281 0
<i>Pagurus longicarpus</i> Say.	10	19 9	40+
Shrimp:			
<i>Palaemonetes carolinus</i> Stimpson	10	4 8	5 0
Crabs:			
<i>Callinectes sapidus</i> Rathbun	14	34 4	97 3
<i>Eurypanopeus depressus</i> (Smith)	10	36 8	44 3
<i>Libinia dubia</i> H. Milne-Edw.	1	22 8	—
<i>Ocypode albicans</i> Bosc	10	87 5	115 1
<i>Panopeus sayi</i> Smith	10	47 3	67.0
<i>Sesarma cinerea</i> Say	10	32 8	46 8
<i>Uca minax</i> (Le Conte)	9	42 7	51 3
<i>Uca pugilator</i> (Bosc).	10	40 2	40 2
Isopods:			
<i>Ligyda oceanica</i> (L.) ..	10	27 8	42 3
<i>Orchestia platensis</i> Kroyer	10	9 4	9 4
Xiphosuran:			
<i>Limulus polyphemus</i> (L.)	10	63 5+	63 5+

clean glass dishes and allowed to stand on laboratory tables. No determinations of humidity, temperatures, or air currents were made. The results of these tests are shown in Table 2. The types which showed

most resistance were those which were accustomed to spend much time out of water (*Littorina*), and those with heavy protective shells (*Limulus*, *Venus*, *Clibanarius*). In general larger animals survived longer periods of desiccation than smaller. Small arthropods in general showed little resistance (*Orchestia*, *Palaemonetes*).

DISCUSSION

According to Redeke (1922) brackish water animals in Holland may be placed in three groups: (1) *Oligohaline*, which have been largely derived from fresh water and usually live in salinities between 0.2 and 1.9‰; (2) *Mesohaline*, which include many proper brackish water animals which live in salinities between 2.0 and 18.5‰; and (3) *Polyhaline*, which are mostly marine and live in salinities between 18.6 and 31.8‰. Remane (1934) found that fewest species lived at the borderline where fresh water and marine animals met, at salinities of 5-8‰. In his paper on the marine molluscs of the Beaufort region Jacot (1921) points out that there are two groups in regard to distribution: (1) the species that occur on the beach of the open sea and (2) those in the sounds. The former seldom get into the sounds and many of them occur in deep water.

In the experiments described in the present paper in which the ability of various estuarine animals to live in diluted sea water was tested (Table 1) none was found which lived in fresh water longer than in sea water. About seven seemed to be better fitted for brackish water than for either fresh or sea water. Perhaps these are to be looked upon as proper brackish water types. Two of them (*Sesarma*, *Orchestia*) are really burrowing land animals that live at the drift line above high-tide mark, but neither can exist away from the ocean. Three of the others (*Bankia*, *Modiolus*, *Emerita*) are burrowers which usually remain submerged and are only active under water. Another, the oyster, is a sessile animal which is also active only when submerged, and is, as many investigators have shown (Amemiya 1928), adapted to brackish water. The seventh species, *Fundulus heteroclitus*, is an euryhaline type which migrates freely from fresh to salt water, and will survive direct transfer from the sea to fresh water (Sumner 1911). As Annandale (1922) has pointed out many types of marine animals continually attempt to spread from the ocean into estuaries, but comparatively few become permanently established.

Many estuarine types are adapted to live in the silty deposits which are characteristic on bottoms. Many burrow and are able to tolerate

very low oxygen pressures in the water which surrounds them (Cole 1926). Life in the soft bottom has certain advantages: the water in the floor of an estuary often remains more salty than that above (Alexander, Southgate, and Bassindale 1932) when water is flowing out from the fresh water tributaries and oxygen in burrows may at times be maintained at a rather high concentration by seepage (MacGinitie 1935).

Typically marine animals which have crossed the brackish regions of the estuaries and almost or actually entered the fresh waters beyond are types like (1) oysters and barnacles, which are so well insulated by thick calcareous coverings that they can fast for long periods during freshets and feed only when salinities are favorable, and (2) active, euryhaline types such as the blue crab (*Callinectes*) and the top minnow (*Fundulus heteroclitus*). There are certain obstacles which retard or prevent the entrance of marine animals into fresh water. Respiration is more difficult in fresh water (Schlieper 1933). Calcium is essential for certain animals, probably in connection with buffer actions and alkali reserves, and when present in quantity certain fishes can leave the ocean and enter waters that contain it (Breder 1933). Such things as barnacles and oysters may by favorable combinations of circumstances become established and with their rather remarkable powers maintain themselves and grow for a time, but they can probably never become fresh water animals. The inability to carry on successful reproduction is probably the final factor which prevents many marine animals which almost succeed from attaining fresh water permanently.

If one looks in the Beaufort estuaries for types of animals that are leaving land and fresh water to enter the ocean there are few to be found. No mollusc appears to be migrating from fresh water to sea, though some are favorably situated (Polymesoda; van der Schalie 1933). Insects are continually carried down by streams into estuaries and some have become established in the latter, but they are few in numbers compared with those which are found in brackish ponds and marshes. This is probably because insects, though generally progressive and aggressive, are strongly committed to air-breathing through tracheal tubes; and open sounds with strong waves and currents are therefore unfavorable. Insects are invading the sea from land and fresh water (Pearse 1932, 1936) but are to be found in quiet, protected waters. They are favored in this by the fact that ponds and marshes, even quite near to the ocean, often tend to maintain salinities much below that of sea water. In such habitats insects are often associated with typically marine animals.

Many animals appear to have crossed ocean beaches to become

established gradually on land, but few have attained fresh water or land through estuaries (Annandale, 1922; MacGinitie, 1935; Pearse 1936).

SUMMARY

1. Beaufort, North Carolina, is favorably located for the study of estuarine animals, as it is surrounded by banks, sounds, and estuaries.

2. During two summers collecting was carried on and determinations of salinity, temperature, oxygen, and hydrogen-ion concentration were made. Tides at Beaufort usually have a vertical range of 2.5 ft.

3. A list of animals and a few plants collected is given.

4. The toleration of 45 estuarine species for dilutions of sea water was tested. Among these none lived longer in fresh water than in sea water, seven appeared to be adapted to brackish water, and the remainder were marine.

5. The toleration of 25 estuarine animals to desiccation was tested. Types which proved to be most resistant were those accustomed to spend much time out of water and those with thick protective shells.

6. Few marine animals appear to have migrated through estuaries to attain life on land, but many have gone from the ocean directly over beaches. Difficulties encountered in estuaries are concerned chiefly with salinity, respiration, and reproduction.

7. Some estuarine animals show adaptations for burrowing and toleration of low oxygen concentrations; others are active and euryhaline.

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PLATE 15



FIG. 1. POND IN PEAT; OPEN GROUNDS

FIG. 2. HARLOWE CREEK CANAL



FIG. 3. MULLET POND
FIG. 4. ALONG LENOXVILLE ROAD

RACIATION IN STENIRIDIA ANDREWSI HARRIS, A SUPPLEMENT TO SPECIATION IN STENIRIDIA

By J. M. VALENTINE

PLATE 17

It has been very tactfully and pleasantly brought to the author's attention by his colleagues that he has "committed a synonym." In his recent paper, *Speciation in Steniridia*,* he has unwittingly placed Casey's *Steniridia amplicollis* (1920) and *reflexa* (1924) as variants of *Steniridia andrewsi montana*, new subspecies. The excuse, admittedly a poor one, is that arbitrary taxonomy was for the time obscured by much more significant biological data. Though the author rebels, on pragmatic grounds, against the obligation of accepting an aberrant individual as the type of an important and widely ranging geographical race, he finds it his painful duty to compromise in order to comply with a necessary rule of nomenclatorial priority.

Amplicollis obviously does not typify the great *andrewsi* population inhabiting the mountains of northwestern North Carolina and southwestern Virginia, which race, for the sake of simplicity, the writer proposed (1935) to embrace with all its possible subraces under one name—*montana*. Casey's type specimen would cause a never-ending source of annoyance and false hope among future students who, comparing their more normal examples of *andrewsi* with it, might easily be tempted to describe, in the Caseyan manner, any number of "new" things within the species. Rather than run this risk, it seems better to split the existing subspecies of *andrewsi* into a secondary series of races, thus repeating the analysis of the species at a level of greater dichotomy. A certain degree of simplicity will doubtless be sacrificed by this process, yet at the same time a more complete picture will be gained of the relationship between genetic trends and the topographic features which act as barriers to and vehicles of distribution.

In *montana*, there is every gradation in pronotal contour from the straight-sided, narrowly margined pronotum close to that of race *germari*, to the condition of widely flaring rounded margins seen in

* See bibliography.

amplicollis, an extreme case. It is true, however, that the widest margins are typical of examples from the somewhat isolated Black Mountain Range of North Carolina. It is likewise true that narrower margins are characteristic of the *andrewsi* fauna from the type locality of *montana* and from the adjoining Grandfather Mountain, Avery Co., North Carolina. Given the existing type material, these subraces should be differentiated. If *they* are subspecifically handled, it will be necessary to undertake a parallel splitting within other forms of *andrewsi* already described as subspecies. Thus *germari* and *parvitorsalis* must also undergo geographico-genetic subdivision. To accomplish this, other external characters such as general proportions, color, sculpture and male tarsal scalation must be brought in to supplement the variable pronotal contour.

A knowledge of relatively exact localities is a primary prerequisite to proper identification of geographic races; for this reason it seems unnecessary and misleading to adopt the key method of separating such forms. We are dealing with freely crossing segregates belonging to *one genitally constant species* whose external characters vary with locality and environment; we are not herein concerned with stable, true-breeding species of drastically distinct genitalic equipment.

Additional data acquired subsequent to the publication of *Speciation in Steniridia** are included in the following revision of *Steniridia andrewsi*. The same technique in measurement and in calculating indices of parts will be followed here. For details and explanations the reader is referred to the first paper.

Steniridia andrewsi may now be divided into the following groups:

- I. *Group andrewsi* s. str.: Relatively large, brilliant forms with comparatively smooth sculpture, narrow, much reflexed pronotal margins and restricted development of the scaly pads on the male anterior tarsi (tarsal index not over .53).
Chorology: Low altitudes; piedmont section of central North Carolina.
 1. *Steniridia andrewsi andrewsi* Harris.
- II. *Group montana*: Medium sized, relatively dark forms with deeper, more irregular sculpture, wider pronotal margins and in the male the most extensive anterior tarsal pad development (tarsal index .69-.82).
Chorology: Intermediate to fairly high altitudes, mainly the

* See bibliography.

former; the Appalachian Mountains from southwestern Virginia southwest through North Carolina and along the Tennessee line to the French Broad River.

2. *Steniridia andrewsi montana* Valentine.

3. *Steniridia andrewsi amplicollis* Casey.

III. *Group parvitorsalis*: Similar to the last but with the plantar pads of the male front tarsus reduced in size (tarsal index .60-.69).

Chorology: Intermediate to high altitudes, mainly the former; the extreme southwestern end of the Appalachian Mountains: in North Carolina, west of the French Broad River and south of the river system of the Little Tennessee; in South Carolina, Georgia and Tennessee, those mountainous portions immediately adjacent to the above area in North Carolina.

4. *Steniridia andrewsi parvitorsalis* Valentine.

5. *Steniridia andrewsi nantahalae* n. ssp.

6. *Steniridia andrewsi saludae* n. ssp.

IV. *Group darlingtoni*: Medium to small, very dark, deeply, irregularly sculptured races of variable form and tarsal pad development (tarsal index .62-.70).

Chorology: Intermediate to very high altitudes in the Smoky Mountains of North Carolina and Tennessee north of the river system of the little Tennessee.

7. *Steniridia andrewsi darlingtoni* Valentine.

8. *Steniridia andrewsi barksdalei* n. ssp.

V. *Group germari*: Medium to large, relatively brilliant forms having locally irregular sculpture, narrowly margined, straightsided (posterior to lateral angle) pronota and reduced male tarsal pad development (tarsal index .47-.69).

Chorology: Low to intermediate altitudes; within the Appalachian Plateau over that area south of the glacial line drained by the Ohio and Tennessee Rivers and their tributaries.

9. *Steniridia andrewsi germari* Chaudoir.

10. *Steniridia andrewsi mutabilis* Casey.

11. *Steniridia andrewsi waldensia* Valentine.

1. *Steniridia andrewsi andrewsi* Harris

Cychrus andrewsii Harris, 1839

Irichroa andrewsi Harris—Casey, 1920

Steniridia andrewsi Harris—Casey, 1924

Type: ♀, lost.

Neotype: ♀, U. S. National Museum; J. M. Valentine, collector, 1934.

Neotype locality: Chapel Hill, Orange Co., North Carolina.

An additional record from Climax, Guilford Co., North Carolina, extends the range of the type race forty miles to the west.

2. *Steniridia andrewsi montana* Valentine, 1935

Holotype, allotype: ♂, ♀, U. S. National Museum; J. M. Valentine, collector, 1934.

Type locality: Beech Mountain, Avery Co., North Carolina.

Among the males of this race are found individuals exhibiting a greater degree of anterior tarsal dilation and pad development than is characteristic of any other known *Steniridia* (tarsal index .70-.82).

The range includes the mountains of northwestern North Carolina (Avery, Watauga Cos.) and probably the extension of the same high mountain chain in southwestern Virginia (Grayson Co). Examples from further north at Mountain Lake, Giles Co., Virginia, possess pronota which, in their narrowness, tend toward some race of the *germari* group. However, the male tarsi (index .70-.77) are of the *montana* type; for this reason the colony had best be included in *montana* whose range doubtless overlaps that of a subrace of *mutabilis* along the New River. A single female, taken by the author at Little Switzerland, McDowell Co., North Carolina, establishes the southernmost record for the race.

3. *Steniridia andrewsi amplicollis* Casey

Irichroa violacea amplicollis Casey, 1920

Irichroa andrewsi reflexa Casey, 1924

Steniridia andrewsi montana Valentine, 1935

Steniridia andrewsi montana var. *amplicollis*

Casey—Valentine, 1935

Type, paratype: ♂, ♀, U. S. National Museum; Wm. Beutenmuller, collector, 1912.

Type locality: Black Mountains, North Carolina.

Type ♂: length 23 mm.; width 8.8 mm.; head index .45; pronotal index .78; elytral index .68; abdominal index .42; tarsal index .715. *Color*: violaceous above with faint blue reflections.

Topotypes, Black Mountains and Mt. Mitchell, Buncombe and Yancey Cos., North Carolina: seven ♂s: length 20.5–22.2 mm.; width 7.6–8.6 mm.; head index .43–.46; pronotal index .76–.83; elytral index .66–.72; abdominal index .42–.49; tarsal index .69–.79. Four ♀s: length 22.7–23.6 mm.; width 8.9–9.5 mm.; head index .42–.44; pronotal index .78–.80; elytral index .67–.75; abdominal index .42–.47. *Color*: violaceous above with variable blue reflections, less evident on elytra; below black, epipleurae very faintly violaceous; tarsi dark brown.

The type specimen of *amplicollis* is an exaggerated form of its race having pronotal margins of probably maximum extent. The paratype is more normal, being essentially similar to the above topotypes. *Amplicollis*, more or less isolated on the Black Mountain Range, has departed from its closest relative, *montana*, in the following overlapping characters: the color is more violet and less blue; the pronotum is more broadly margined, especially at the rounded lateral angles, and possesses on the average fewer basal punctures, often lacking them entirely; the male tarsi have, on the average, smaller first plantar pads.

Andrewsi reflexa Casey (Plate 17, fig. 1), likewise from the Black Mountains, is merely a large (24.6 mm.) female of *amplicollis*, identical in all other respects with topotype material of the latter.

Concerning the habits of *amplicollis*, Beutenmuller (1918) writes: "I have never taken it on all my trips on the extreme summit or higher parts of the slopes."

4. *Steniridia andrewsi parvitorsalis* Valentine, 1935

Type: ♂, U. S. National Museum.

Type Locality: Clayton, Rabun Co., Georgia.

With the separation of *nantahalae* from *parvitorsalis*, the range of the latter race is now restricted to the mountains chiefly of Hiwassee and Little Tennessee drainage including the Blue Ridge of northeastern Georgia and the Unaka Mountains of southwestern North Carolina and southeastern Tennessee. It lives in the relatively low mountains flanking the main Appalachian chains.

5. *Steniridia andrewsi nantahalae* Valentine, n. ssp.*Steniridia andrewsi parvitorsalis* Valentine, 1935*Type*: ♂, U. S. National Museum; J. M. Valentine, collector, 1931.*Type locality*: Cashier's, Jackson Co., North Carolina; 3500 ft.*Type* ♂: length 20.0 mm.; width 8.1 mm.; head index .45; pronotal index .80; elytral index .72; abdominal index .47; tarsal index .69. *Color*: above, dark violaceous with blue reflections especially on the margins; below, black, epipleurae faintly purple; tarsi brown, not dark. *Head*: normal. *Pronotum*: margins widely flaring anterior to obtuse but evident lateral angles; elongated, straight-sided and tapering posterior to lateral angles; lateral and anterior margins and basal impressions marked with confused punctures. *Elytra*: normal in form; discal costae interrupted half way to base, obliterated over apical fourth (Plate 17, fig. 2).*Paratype*: ♂, Jocassee, Oconee Co., South Carolina; Whitewater River 2200 ft.; D. Dunavan, collector. Length 19.8 mm.; width 7.9 mm.; head index .46; pronotal index .85; elytral index .72; abdominal index .48; tarsal index .69. *Color*: above, dark violaceous.

The high, southern Blue Ridge Mountains at the point where they are joined by the Nantahala and Cowee Mountains are the home of typical *nantahalae*. Being a high altitude form of *parvitorsalis*, it differs from the latter chiefly in those characters which are correlated with elevation: it is smaller, darker, and exhibits a more extensive interruption of elytral costae. However, the greater development of the male's anterior plantar pads is a character which probably is not so closely related to environment but serves rather to connect, genetically, the two groups: *parvitorsalis* and *montana*.

A series collected by G. Beyer in the "Blue Ridge Mountains, North Carolina" and measured by the author (see bibliography) is identical with the type. Another series of three females taken at Rocky Bottom, Pickens Co., South Carolina, by D. Dunavan and O. L. Cartwright agrees with the type in all respects save that these examples have a more angular pronotum and are of the size of *parvitorsalis*: length 21.5-23.2 mm.; width 8.3-9.4 mm.; head index .42-.46; pronotal index .84-.89; elytral index .69-.72; abdominal index .47-.51. A single female taken at Sunburst, Haywood Co., North Carolina, by O. L. Cartwright represents the Pisgah Ridge segregate of *nantahalae*: length 20.7 mm.; width 8.0 mm.; head index .48; pronotal index .82; elytral

index .69; abdominal index .46. The lateral margins of the pronotum of this specimen also are more angular than in the type.

6. *Steniridia andrewsi saludae* n. ssp.

Type: ♀, U. S. National Museum: W. F. Fiske, collector, 1903.

Type locality: Melrose, Polk Co., North Carolina.

Type ♀: length 20.8 mm.; width 8.4 mm.; head index .42; pronotal index .87; elytral index .73; abdominal index .50. *Color*: above, dark violaceous; below, black, epipleurae faintly purple; legs dark brown (may be due to slight immaturity), tarsi not perceptibly lighter. *Head*: normal. *Pronotum*: narrow, straight-sided posterior to rounded lateral angles; margin narrow, abruptly reflexed especially in posterior half; impunctate except in basal impressions. *Elytra*: conspicuously elevated in the humeral region, humeri prominently rounded; costae pronounced, interrupted only over apical fourth of elytra; striae deeply, regularly punctured; epipleurae coarsely, irregularly and profusely punctured (Plate 17, fig. 3).

Until males are found, *saludae* is tentatively included in group *parvitarisalis* because of its geographic proximity to members of the latter. However, it bears an equal resemblance to the piedmont-dwelling, neotypic race of *andrewsi* from which it differs thus: it is smaller, darker (including tarsi) and more convex; its pronotum is narrower, the humeri more expanded and the elytral punctuation coarser throughout. All these are characters which may simply reflect life at higher elevations in the Saluda Mountains where it was taken.

The Saluda Mountains constitute the eastern extremity of the southern Blue Ridge from the main range of which they are almost completely separated by the French Broad river system.

7. *Steniridia andrewsi darlingtoni* Valentine, 1935

Holotype, allotype: ♂, ♀, Museum of Comparative Zoology; P. J. Darlington, Jr., collector, 1930.

Type locality: State Road to Newfound Gap, Sevier Co., Tennessee; 3500 ft.

Darlingtoni seems to be typical of the high mountains of the southwestern end of the Great Smoky Range along the North Carolina-Tennessee line. Here it ascends to the summit cloud forests of spruce and balsam. Under these ecological conditions are found the smallest,

darkest specimens of roughest sculpture and narrowest pronotal margination. A female, collected by L. Barksdale from the divide running at an elevation of about 4000 ft. from Andrews Bald to Mt. Buckley, has the following characters: length 19.5 mm.; width 7.6 mm.; head index .42; pronotal index .91; elytral index .69; abdominal index .47. *Color*: head and pronotum blue-green-black; elytra violaceous-black; tarsi almost black. The pronotum is very narrow and a lateral band of vermicular costal interruption extends to within one third of the distance to the elytral base.

8. *Steniridia andrewsi barksdalei* n. ssp.

Holotype, allotype: ♂, ♀, U. S. National Museum; L. Barksdale, collector, 1934.

Type locality: Mt. Guyot, Swain and Haywood Cos., North Carolina; about 3500 ft.

Holotype ♂: length 20.9 mm.; width 8.1 mm.; head index .44; pronotal index .78; elytral index .71; abdominal index .44; tarsal index .69. *Color*: above, dark blue with violaceous reflections; below, black, epipleurae faintly violet; tarsi dark brown, anterior ones paler. *Head*: median clypeal groove deeply impressed. *Pronotum*: anterior lateral margins broadly, evenly rounded, lateral angles almost obliterated, posterior lateral contour slightly concave; margins and basal disc impressed with shallow, confused punctures. *Elytra*: humeri reduced; sculpture coarse but regular, punctation wide, deep, causing costae to appear distinctly wavy; discal costae locally vermiculate over apical half, interrupted over apical fifth (Plate 17, fig. 4).

Allotype ♀: length 23.2 mm.; width 9.0 mm.; head index .44; pronotal index .81; elytral index .69; abdominal index .45. *Color*: similar to type but more violaceous; all tarsi dark brown. In body form and sculpture it is identical with the type.

This race seems to hold an intermediate position between *nantahalae* and more northerly races of *andrewsi*. The deeply pitted elytra of beaded appearance and more extensively scaled male front tarsus serve to distinguish it from the former while sculpture, particularly that of the pronotum, and pronotal contour are the principle features differentiating it from group *montana*.

A geographical connection with *nantahalae* probably exists along the Balsam Mountain ridge which joins the northeastern end of the Smoky Mountains, where *barksdalei* is found, with the Tennessee and Pisgah Ridges of the southern Blue Ridge system.

It is a pleasure to name the subspecies after Lane Barksdale who collected the only two specimens seen by the author.

9. *Steniridia andrewsi germari* Chaudoir, 1861

Type: ♀, collection of R. Oberthür-Rennes.

Type locality: Tennessee.

Specimens from Pineville, Bell Co., in the extreme southeastern corner of Kentucky, in all likelihood represent typical *germari* since this locality lies in the Cumberland Mountains which extend well into Tennessee. A much larger race, *waldensia*, inhabits the Walden Ridge to the south. All other important mountain ranges in eastern Tennessee are represented in the author's collection by examples of *andrewsi*, none of which corresponds to the original description of *germari*. It would seem probable, therefore, that somewhere in the Cumberland Mountains of Tennessee lies the true type locality of this insect. Added evidence in support of this view is that specimens from both the Kentucky and Virginia portions of the Cumberlands agree perfectly with Chaudoir's description.

A diagnosis of the Pineville specimens follows: ♂: length 20.5 mm.; width 7.9 mm.; head index .45; pronotal index .86; elytral index .71; abdominal index .45; tarsal index .58. ♀: length 24.4 mm.; width 9.8 mm.; head index .44; pronotal index .87; elytral index .73; abdominal index .47. *Color* of both: violaceous above, head and pronotum darker, former almost black with blue-green reflections. These specimens resemble examples from Lee Co., Virginia and from Harlan Co., Kentucky (Plate 17, fig. 5), in having elevated, expanded humeri. In agreement with this observation is the following statement made by Chaudoir in reference to *germari*: "épaules qui sont arrondies comme dans le *viduus*." The pronotal margins are wider than in the more northerly race heretofore identified as *germari* and the sides and base of the pronotum are more coarsely and densely punctured.

10. *Steniridia andrewsi mutabilis* Casey

Irichroa mutabilis Casey, 1920

Irichroa mutabilis longicollis Casey, 1920

Irichroa mutabilis modulata Casey, 1920

Steniridia andrewsi germari Chaudoir-Valentine, 1935

Type, paratypes: 5♂s, U. S. National Museum; T. N. Brown (?), collector.

Type locality: Uniontown, Fayette Co., Pennsylvania.

Type ♂: length 23.6 mm.; width 8.8 mm.; head index .43; pronotal index .97; elytral index .69; abdominal index .42; tarsal index .64.

Color: above, head blue-black, pronotum and elytra violaceous with blue reflections (Plate 17, fig. 6).

Topotypes, chosen from a large series to illustrate the greatest range of variation within the series which includes two male paratypes of *mutabilis*, the male type and two female paratypes of *longicollis* and the unique male type of *modulata*: 7 ♂s: length 20.7–24.2 mm.; width 8.2–9.0 mm.; head index .43–.48; pronotal index .88–.97; elytral index .67–.72; abdominal index .40–.45; tarsal index .60–.71. 5 ♀s: length 24.2–27.2 mm.; width 9.2–10.7 mm.; head index .44–.46; pronotal index .90–1.02; elytral index .68–.70; abdominal index .43–.46. *Color:* above, violaceous with blue-green reflections, especially on head, elytra often with aeneous cast, sometimes almost black; below, black, epipleurae faintly purplish; tarsi rather dark brown.

The Uniontown colony of *andrewsi* differs from typical *germari* in averaging less convex and in having a narrower pronotum, less expanded humeri and, in the male, a more extensive anterior tarsal pad development. The sixth and tenth elytral costae are usually conspicuously vermiculate. These distinctions make it necessary, under the standards of the present analysis, to recognize one of the three Casey names applied to examples of this colony. By page priority, *mutabilis*, given by Casey as a specific name, becomes valid as a subspecific name while his subspecies *longicollis* and *modulata* remain in synonymy as mere variants within the norm.

Mutabilis and its subraces (hardly worthy of nomenclatorial differentiation) seem to be restricted to the Ohio valley and to the highland plateau drained by the tributaries of this river. Specimens from the Monongahela valley at Fairmont, Marion Co., West Virginia, forty miles south of the type locality, possess pronota which are even more elongate (index as high as 1.05) than in the type colony; examples from an area in the Ohio basin including Cincinnati, Ohio, and Frankfort, Franklin Co., Kentucky, are all quite similar to *mutabilis* though the male specimens have tarsi as in *germari* proper. A single female taken by the author at Gawley Mountain, Fayette Co., West Virginia, 2600 ft., closely resembles *mutabilis* except for the sculpture which, in its coarseness, appears to exhibit the influence of higher altitude, and the elytral margin which is wider.

11. *Steniridia andrewsi waldensia* Valentine, 1935

Cychnus germari Chaudoir-Liebeck, 1899

Holotype, allotype: ♂, ♀, Philadelphia Academy of Sciences; H. A. Pilsbry, collector.

Type locality: Sawyer's Springs, Hamilton Co., Tennessee.

The two examples comprising the type series of this race are the largest *andrewsi* yet recorded. They were taken near the summit of the southern end of Walden Ridge at about 1500 ft. elevation where conditions of relative dryness prevail.

The male tarsal equipment and size combine to place *waldensia* in group *germari* in spite of the more expanded antero-lateral pronotal margin which character links it, perhaps, with *parvitorsalis*.

CONCLUSIONS

1. A geographic or ecological race (subspecies) within a genitally constant species of *Steniridia* (such as *andrewsi*) contains the entire, local population of that species as distinguished from neighboring and related segregates on the basis of diagnostic, though overlapping characters of size, color and sculpture.

2. Races adapted to lowland conditions have followed the river systems over relatively great distances and therefore have much more extensive ranges than races of upland habitat (*andrewsi* s. str., *mutabilis*, etc.).

3. Those races which prefer higher altitudes are ecologically limited to the particular range in which they have evolved. For these, the mountain chains rather than the drainage systems are the vehicles of distribution. Consequently, the higher the altitude preferred the more limited the range of the race and the more complete the isolation factor (*nantahalae*, *darlingtoni*, etc.).

4. In races of *Steniridia andrewsi*, as in species of *Steniridia*, size, color and sculpture may be correlated with the respective altitudes at which they are found; mean temperature and especially moisture conditions are indicated as factors influencing these characteristics.

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Errata in the above paper:

Page 351: second line from bottom, for Dowell read McDowell.

Page 354: sixteenth line from top, for "*mutabilis*" read "*modulata*."

Page 358: third line from bottom, omit *andrewsi*.

EXPLANATION OF PLATE 17

(4 ×)

- Fig. 1. *Scaphinotus Steniridia reflexa* Casey = *andrewsi amplicollis* Casey. Type ♀. Black Mountains, North Carolina. W. Beutenmuller, 1912.
Fig. 2. *Scaphinotus Steniridia andrewsi nantahalae* n. ssp. Type ♂. Cashier's, Jackson Co., North Carolina. J. M. Valentine, 1931.
Fig. 3. *Scaphinotus Steniridia andrewsi saludae* n. ssp. Type ♀. Melrose, Polk Co., North Carolina. W. F. Fiske, 1903.
Fig. 4. *Scaphinotus Steniridia andrewsi barksdalei* n. ssp. Type ♂. Mt. Guyot, Swain and Haywood Cos., North Carolina. L. Barksdale, 1934.
Fig. 5. *Scaphinotus Steniridia andrewsi germari* Chaudoir. ♀. Pine Mountain, Harlan Co., Kentucky. W. Stone, 1921.
Fig. 6. *Scaphinotus Steniridia andrewsi mutabilis* Casey. Type ♂. Uniontown, Fayette Co., Pennsylvania. T. N. Brown (?).



1



2



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6

NOTES ON THE HISTORY OF THE GERM CELLS IN THE TOADFISH (OPSANUS TAU)*

By EUGENE P. ODUM

PLATES 18 AND 19

It seems to be definitely shown that germ cells are segregated at an early stage of development in many species of vertebrates and invertebrates. In the vertebrates so-called primordial germ cells are usually described as being first distinguishable, by their large size and rounded shape, in the peripheral endoderm of the embryo at a time when the germ layers have recently formed and are found to migrate through the developing tissues to a place under the dorsal peritoneum ventral to the Wolffian ducts, in which position the gonads develop. Concerning the subsequent history of these cells, especially as to whether or not they become the definitive germ cells, there is, however, a great diversity of observations. Among recent contributions to this question in the case of fish, Okkelberg (1921: brook lamprey), Hann (1927: *Cottus bairdii*), Goodrich *et al.* (1934: *Lebistes reticulatus*) have presented evidence that the primordial germ cells do form definitive germ cells. Hann's work is especially interesting because of its completeness. After a study of adult seasonal stages as well as embryos he concluded that the primordial germ cells were the source of all the definitive germ cells throughout life. In contrast Essenberg (1923) found that the primordial germ cells in *Xiphophorus* mostly degenerated at the time of sex differentiation, to be replaced by germ cells arising from the peritoneal epithelium of the ovarian cavity and sperm duct, respectively. Wolf (1931: *Platypoecilus maculatus*) and Butcher (1929: lake lamprey) found that primordial germ cells, although not undergoing degeneration, were supplemented with germ cells of peritoneal origin. Foley (1927) studying adult testis only of *Umbra limi* found abundant transition stages be-

* The material for this research was collected and reared in the Beaufort (N. C.) Laboratory of the U. S. Bureau of Fisheries and the author wishes to express his indebtedness to the Commissioner of Fisheries, Hon. Frank T. Bell, and the Director of the Laboratory, Dr. Herbert F. Prytherch, for the facilities there provided. Also to Professor H. V. Wilson for guidance and criticism during the course of the work.

tween stroma cells and germ cells. Similar lack of uniformity is found in the literature of other vertebrate groups.

Sink (1912) in a short paper published the results of a study of the germ cells of early embryos of the toadfish (*Opsanus tau*) up to 8 mm. in length. He was unable to find primordial germ cells earlier than in a 3.5 mm. stage at which time they were located in the endoderm of the tubular gut. In slightly larger specimens they were located in the mesentery and under the Wolffian duct. In the 8 mm. stage a gonad had formed as a small sac containing the germ cells.

Since the toadfish gave promise of being a favorable form, a study was undertaken of later embryos and larvae in an effort to determine, if possible, the fate of the early segregated germ cells and the origin of the definitive cells.

The following observations are based on a study of a series of embryos from 6 mm. body length to larvae of about 23 mm. length. The series covers the development of the gonad through the period of sex differentiation.

Embryos 6 mm. in length. The body of the embryo is well formed although the yolk sac is still relatively enormous; the embryo is perched upon a large egg-shaped yolk sac. The germ cells are conspicuous at this stage (figs. 1a, 2). They lie under the dorsal peritoneum just ventral to the Wolffian ducts. The distribution of the germ cells is remarkably uniform; they lie on each side in a practically continuous stripe one cell thick and from one to three cells wide. A longitudinal section of this stage (fig. 1a) shows that the germ cells are closely appressed one behind the other. A gonad, as an organ projecting into the coelom, has not yet formed.

The germ cells occur over a length of 0.75 mm. in the posterior region of the embryo, extending anteriorly to the region of the liver and posteriorly almost to the end of the coelomic cavity. There are approximately 250 germ cells in the particular embryo studied.

Close examination of these cells reveals that they possess the typical characteristics of primordial germ cells as described for the vertebrates. They are large and rounded in shape, rivaled in size only by the differently shaped cells of the gut epithelium. They stain lightly as compared with the mesenchyme cells. The nucleus is spherical and contains a large darkly staining nucleolus, sometimes two, and numerous chromatin granules. Large chromatin granules are often aggregated just inside the nuclear membrane, smaller ones are scattered through the central portion of the nucleoplasm; a reticular network is present.

The cytoplasm stains lightly with eosin. The yolk granules possessed by primordial germ cells at segregation have apparently been absorbed. Material was not at hand for examination of earlier stages, but in view of Sink's work on the early stages and the general occurrence of similar cells in the early stages of many fish, there is no reason for supposing that these cells in the 6 mm. embryos are anything but primordial germ cells which have originated in the endoderm and migrated to the place they now occupy. We wish now to see, if possible, what part they play in the development of the sex glands.

Embryos 7 mm. in length. Although the stage is but slightly larger than the last there has been considerable activity in the genital region. In the middle part of this region, a conspicuous gonad has formed consisting of a peritoneal sac containing germ cells (fig. 3, just below the Wolffian duct). In the anterior and posterior portions of the genital stripe or ridge gonad formation has not progressed so far, and from a study of these regions we get an idea of the precise manner in which the gonadial fold forms. The cells of the peritoneal membrane on either side of the germ cells apparently divide rapidly, pushing the germ cells and their peritoneal covering out into the coelom. There is no evidence which indicates that any of the mesenchyme cells lying dorsal to the germ cells enter the gonad; the two layers forming the neck or "mesentery" of the gonad are from the beginning appressed closely. The gonad is then formed only from peritoneal cells and germ cells, the former active, the latter passive. The stroma cells which later occupy the interior of the gonad along with the germ cells originate from the peritoneal covering.

It has been suggested that the germ cells perhaps are not passive during the formation of the gonad, but exert some sort of activating influence on the peritoneal cells. Such might conceivably be the case, but the immediate presence of germ cells is not necessary for the formation of a gonadial fold in the toadfish. Anterior and posterior to the germ cell region the gonadial fold forms although there are no germ cells in the fold or in the neighborhood.

Embryos 9 mm. in length. The gonad now appears in section as a club-shaped organ (fig. 4) somewhat deeper dorsi-ventrally than in the 7 mm. embryo. The germ cells, to all appearances, remain unchanged, although their number in the particular embryo studied is only about 171. They show no indication of degeneration or division. The stroma cells of the gonad, however, have increased and now make up a large part of the interior of the gland. The germ cells are in consequence no

longer closely appressed but are surrounded by stroma cells. In front of this region containing germ cells, the gonad for a distance of about $450\ \mu$, though well formed, contains no germ cells. Also for about $170\ \mu$ behind the germ cell region, a gonadial fold has formed with no germ cells.

Embryos 11 mm. in length. Sections of this stage (fig. 5) show a similar condition of the gonads. The stroma cells have, however, increased somewhat; dividing stroma cells are seen. The gonadial mesentery has become thinner. The germ cells are somewhat smaller, certainly, in some cases; the nucleus is still of the same character, but the cytoplasm is more limited, as if the pressure of the stroma has caused a decrease in the size of the cell.

The length of the gonadial fold is now about 2 mm., and the germ cells number about the same as in the preceding stages (actual count 220). The anterior $500\ \mu$ and the posterior $210\ \mu$ of the gonads contain no germ cells. In the middle region the germ cells are now quite scattered in the more abundant stroma. A longitudinal section of the gonad shows that the germ cells are no longer closely appressed, nor quite uniformly distributed. Stroma intervenes between them.

Embryos 15 mm. in length. Sex differentiation has taken place in this stage. In the males the extreme posterior portion, and this portion only, of the gonadial fold enlarges, and a slit, the sperm duct, shows distinctly in the stroma (fig. 6).

In the case of the females, the gonad along the whole length of the gonadial fold was found to have increased in size without an especially great enlargement in the posterior region. A groove or fold, the first indication of the formation of the ovarian cavity, is to be found on the external side of the anterior part of the gland (fig. 7). Also changes in the histological appearance of the interior indicate that active reorganization of the gonad is in progress.

Embryos 15 mm. in length, male. The remarkable feature in the male gonad is that the portion which enlarges and differentiates (fig. 6) is so short (about $90\ \mu$ in length), while the rest of the gonad, instead of becoming larger, is, if anything, smaller than before. There are no germ cells in the enlarged portion, nor were there any in this general region during earlier stages. Furthermore, the germ cells in the small part of the gonad in which they are present are much smaller than before, roughly about half as large as those in the 6 mm. stage. The cytoplasm of the cell is quite restricted as compared with the earlier condition. The number of germ cells is also smaller. One embryo contained about

98 cells, another 106. The most anterior region as before contains no germ cells.

Embryos 15 mm. in length, female. Sections of the female embryo make it evident that extensive reorganization is in progress over the whole length of the gonadal fold (figs. 7, 1d). It appears that a change is taking place both in the primordial germ cells and the stroma of the gland. In the first place the germ cells are fewer and smaller, the diminution in size being due to a decrease in the cytoplasm (as is the case with the male of this stage and to some extent in the 11 mm. embryos). Furthermore, many of the cells have an abnormal appearance. In a great many cases the cytoplasmic body is irregular and separated by a considerable space from an outer wall made up of stroma cells (figs. 1b, 1c, 7). In a cell or two the nucleus appears to have divided into irregular portions and there is no trace of a nucleolus. In most cases the nucleus is found to have become smaller and more deeply stainable. The changes would seem to be degeneration changes, indicating final disappearance of the primordial germ cells.

The stroma itself seems to be undergoing extensive reorganization. Formerly (fig. 5) it presented the appearance of a tissue in which the nuclei were closely crowded and the limits of the cytoplasmic bodies not definitely observable. Now the stroma in one and the same gonad presents quite different appearances in different regions. In the more posterior region (fig. 7) the dense tissue of the earlier stage has been transformed into loose mesenchymatous tissue with plenty of watery space between the cytoplasmic areas. In the more anterior region (fig. 1d) the nuclei are spaced distinctly further apart than in the earlier stage and the internuclear substance is certainly very largely cytoplasmic. This change is of great interest in view of the pictures presented by the gonads in older stages (figs. 10, 11, 12). It strongly suggests that stroma cells have begun to transform into germ cells.

The count of the primordial germ cells in one embryo is about 116, fewer than formerly. Also, the cells have become still more scattered, so much so that several consecutive sections often show no germ cells. A large blood vessel has formed in the upper center of the gonad.

Embryos 18 mm. in length, male. The enlargement of the extreme posterior portion of the gonadal fold to form a testis which was observable in the 15 mm. embryo, male, has continued. Moreover, the right and left testes have fused so that we now have a single very large gonad lying under the immense urinary bladder (fig. 8, u. b.) The sperm ducts of the originally paired testes are to be seen near the center of the gland.

Also, secondary ducts leading in general towards the primary ducts have formed by dehiscence in the stroma. At the periphery, the cells have begun to aggregate to form masses suggestive of the cysts or tubules of the adult testis. The remarkable fact, as before, is the absence of germ cells in the enlarged portion, which is still short (about $220\ \mu$ long) as compared with the total length of the fold, and contains only a mass of stroma cells.

As before, the much longer anterior portions of the gonadal folds, containing the primordial germ cells, are quite small in transverse section (fig. 9). The part immediately anterior to the enlarged portion is the smallest of all. The gut is here enormous, and the gonad is greatly flattened.

The germ cells themselves (fig. 9) have the same general appearance as in male embryos of the preceding stage. They are small and with a darkly staining nucleus. They are, however, sometimes found in nests (fig. 9) indicating that some division is taking place, whereas formerly stroma intervened between the cells. A total of 312 germ cells was counted in one embryo indicating an increase. A small blood vessel occurs at the base of the mesentery.

The extreme anterior portion of the gonadal fold in the region of the liver contains no germ cells and is apparently being absorbed. It is very small and the mesentery very thin. The total antero-posterior length of the gonadal fold is about 2.8 mm.

Embryos 18 mm. in length, female. The difference between male and female gonads at this stage is profound. The whole middle and anterior portions of the gonad, instead of being quite small, have enlarged two or three times to form an ovary of good size (figs. 10, 11, 12). Furthermore, the ovarian cavity is now conspicuous. In the more posterior portion the formation of the cavity has just begun (fig. 10). Two folds appear on the external surface, one, which had already appeared in the 15 mm. stage, at the base of the mesentery, the other projecting over the lower surface of the ovary. Progressing anteriorly the ovary becomes somewhat wider and the dorsal fold is found to have grown down and the ventral fold up (fig. 11). In the anterior region (fig. 12), the two folds have met and fused, thus forming an ovarian cavity crescentic in transverse sections.

Whatever be the exact nature of the extensive reorganization which was described for the preceding stage, the result is striking. We now have a long gonad in which the germ cells number over 2,000 and are everywhere abundant. They form nests in the ovary of 5 to 10 or

more cells, three or four nests appearing in a section (fig. 11). They thus occupy practically the whole interior of the gonad. The stroma is restricted to the periphery of the organ, a few cells which mark the boundaries of the germ cell nests, and cells that have aggregated around the blood vessel which has shifted to the mesial side.

The cell boundaries of the individual germ cells are in most cases distinct, although sometimes difficult to make out. Otherwise the cells resemble the typical primordial germ cells though smaller. The chromatin granules in the nucleus are also smaller and more evenly distributed.

Posteriorly, the ovaries are continuous with a median mass of stroma concerned with the formation of the oviduct.

Embryos 21 mm. in length, male. They are much the same as the 18 mm. embryos but show certain advances. The gonadal fold is divided into three regions as before: The enlarged posterior part fused with its fellow which has not increased in length ($228\ \mu$), the long middle part (2.4 mm.) containing the whole crop of primordial germ cells, and the anterior, now rudimentary part. The enlarged and differentiated part contains virtually no germ cells. Examination of the developing tubules at the periphery, however, gives some evidence which may indicate a beginning transformation of stroma cells into germ cells. At any rate stroma cells are frequently observed to be aggregated around a cell, the nucleus of which is spherical, showing a nucleolus. The long middle portion of the fold containing the germ cells remains as before relatively very small in transverse section. Where the gut is large the gonads have in some places fused with the mesodermal layers of the intestine, appearing as small projections on the wall of the latter. In the more anterior part the gonad, though small, is lobulated, that is, it bulges out at several points. As was recorded for the 18 mm. male embryos, there is some evidence here of division of the germ cells in that one or two mitotic figures were found. The number of germ cells in a typical case is 226.

Embryos 21 mm. in length, female. Conditions in this stage are quite similar to the preceding. The ovary has become slightly larger. The folding process has proceeded posteriorly until only a small part of the ovarian cavity remains open to the coelom. The germ cells, as before, are packed in the interior of the gland. Some of the germ cells, however, now have vacuoles in them, and sometimes the nucleus is irregular, indicating more degeneration. The oviduct now opens into the cloaca just in front of the opening of the urinary bladder.

Embryos 23 mm. in length, female. The ovary (fig. 13) is now in transverse section much larger than in the preceding stage, and contains, instead of germ cells of uniform small size, germ cells in all stages from small ones to enormous ones that are clearly young oocytes. The small cells occur often in groups. The large ones have a large nucleus containing a large excentric nucleolus and fine chromatin granules. The cytoplasm of the cell stains well with haematoxylin, probably due to the formation of yolk. The intermediate cells of all sizes show that the young ova arise from the small germ cells.

DISCUSSION OF OBSERVATIONS

The stages from 15 mm. to 23 mm. covering the period of sex differentiation are the interesting ones in this study. There is little change in the primordial germ cells up to the time of sex differentiation. As we have seen, in the male, only the posterior part of the gonadal fold enlarges to form the anlage of the testis. This part contains no germ cells. It seems reasonable to conclude, therefore, that if this portion becomes the functional testis or part of it, germ cells must arise from stroma cells. The anterior part of the fold containing all the germ cells behaves in a puzzling way. While the posterior part is becoming greatly enlarged and undergoes considerable differentiation, the anterior part remains small and shows no sex characters (compare figs. 8 and 9). The germ cells in it are small and scattered, although some division is apparently taking place. Whether this part later enlarges to form part of the testis or whether it degenerates could only be determined by study of later stages.

In the female the situation is also puzzling. In the 15 mm. embryos it is evident that a change is taking place; the primordial germ cells are few, scattered, and some are sickly looking. In the next stage (18 mm.) the ovary contains over 20 times as many germ cells. Comparison of fig. 1d and figs. 10-12 is suggestive as to the manner in which these numerous cells might have arisen, that is, from groups of stroma cells. Although intermediate stages would be necessary to settle the point, it seems highly improbable that all of the 2,000 or more germ cells arose from the few scattered primordial cells of the 15 mm. stage. In the latter stage there are mostly stroma cells within the gland with a relatively few germ cells; in the 18 mm. stage the situation is reversed: there are mostly germ cells in the interior of the ovary and relatively few stroma cells. That the numerous germ cells (or at least some of them) of the 18 mm. embryos, apparently a second crop arising from

stroma cells, are true germ cells comes out in comparing sections of this stage with sections (fig. 13) of the 23 mm. female in which all stages of transformation of small germ cells into large oocytes are seen.

Referring to the literature we find that these observations on the toadfish do not correspond with observations on other fish except in a very general way. Other investigators (Okkelberg, Essenberg, Hann, Wolf, and Goodrich *et al.*) found that regardless of their later history the primordial germ cells behaved in a characteristic manner at the beginning of sex differentiation. In the female the cells enlarged; in the male they divided without enlarging to form nests at the periphery of the gland. In the toadfish the primordial germ cells are small and scattered at sex differentiation. The only criterion for sex differentiation is the behavior of the gland itself. No author describes a wholesale transformation of germ cells in the interior of the young ovary as my observations seem to indicate is the case with the female toadfish although Essenberg and Wolf describe the proliferation of germ cells from the epithelium of the ovarian cavity, and Foley the transformation of stroma cells in the adult testis. The entire absence of primordial germ cells in the developing testis (or part thereof) is unparalleled in any of these accounts. Finally, the observations of the toadfish did not bring out anything to indicate a condition of juvenile hermaphroditism as described by Okkelberg in the brook lamprey, or a reversion of sex as Essenberg believed takes place in some cases in *Xiphophorus*.

SUMMARY

(1). The primordial germ cells immediately before the formation of the gonad are evenly distributed along a considerable part of the posterior coelomic region.

(2). The gonadal fold is formed entirely from the peritoneum and includes the primordial germ cells.

(3). The gonadal fold extends anteriorly and posteriorly beyond the region of the germ cells.

(4). As the gonad develops, the primordial germ cells become scattered as a result of the increase in the stroma and the increase in length of the gonadal region. The count of germ cells, allowing for individual variation, is the same for all the stages of the indifferent gonad.

(5). The male sex is indicated by the enlargement of the posterior portion of the gonadal fold, and by the appearance of a sperm duct as a slit in the interior of the gonad.

(6). The female sex is indicated by the appearance of a groove or fold

on the external face of the gonad at the base of gonadal mesentery, and later by the formation of a second fold at the free edge of the gonad. The stroma also undergoes changes. Groups of spherical stroma nuclei surrounded by considerable cytoplasm are seen.

(7). The primordial germ cells of both sexes at sex differentiation are small and greatly scattered. Degeneration of cytoplasm was noted in germ cells of the female. In the male no germ cells are found in the enlarged part of the gonad.

(8). Germ cells in the female increase from about 100 at the 15 mm. stage to over 2,000 at the 18 mm. stage. Although the conclusion is not to be considered final, the evidence indicates that the latter germ cells are a second crop derived from groups of stroma cells. Some of the cells enlarge later to form oocytes; some also show evidence of degeneration.

(9). In the male, the posterior portion of the gonadal fold which contains no germ cells continues to enlarge and differentiate, while the anterior part containing small primordial germ cells remains small and indifferent in character.

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EXPLANATION OF PLATES

(Photomicrographs except fig. 1)

PLATE 18

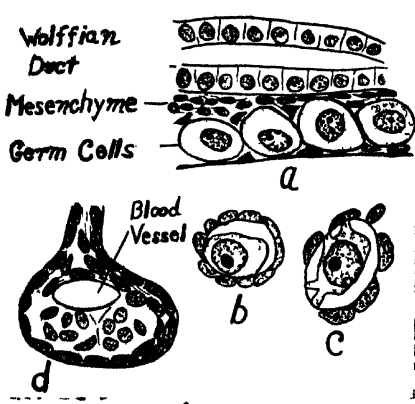
Fig. 1. Camera lucida drawings. Fig. 1a, part of a longitudinal section of 6 mm. embryo showing the uniform arrangement of germ cells before formation of the gonad. Figs. 1b, 1c, oil immersion drawings of primordial germ cells from 15 mm. embryo, female, showing retracted condition of cytoplasm. Fig. 1d, section of gonad of 15 mm. embryo, female, showing spherical

stroma nuclei surrounded by considerable cytoplasm; no primordial germ cells in this section.

- Fig. 2. 6 mm. embryo. Part of transverse section through the trunk region showing large primordial germ cells, three on each side, under the peritoneum ventral to the Wolffian ducts. $\times 143$.
- Fig. 3. 7 mm. embryo. Part of transverse section showing the gonad as a fold of the peritoneum including four primordial germ cells. The Wolffian duct lies dorsal, and a large intestinal vessel ventral to it. $\times 143$.
- Fig. 4. 9 mm. embryo. Transverse section. The gonad is now club-shaped in cross section and contains stroma cells as well as primordial germ cells. $\times 143$.
- Fig. 5. 11 mm. embryo. Transverse section. The stroma has become more abundant within the gonad; the gonadal mesentery has become thin. The space between the mesodermal covering and the columnar epithelium of the gut is an artifact. $\times 143$.
- Fig. 6. 15 mm. embryo, male. Transverse section of posterior part of gonadal fold. The gonad has enlarged and a sperm duct has appeared as a slit in the interior. A part of the urinary bladder (paired at this level) is seen in the corner at the right; the Wolffian duct is above and at the right. Midline of body is towards the left. $\times 143$.

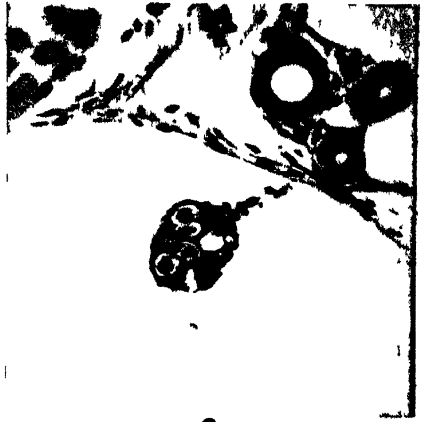
PLATE 19

- Fig. 7. 15 mm. embryo, female. Transverse section of the posterior part of the gonadal fold showing two primordial germ cells and a large blood vessel in the center. The cytoplasm has shrunk in the lower cell. The stroma has become mesenchymatous. The commencing ovarian groove at the base of the mesentery to the right is just perceptible. $\times 143$.
- Fig. 8. 18 mm. embryo, male. From a transverse section. The greatly enlarged posterior part of the fused gonadal folds, forming the (double) testis, lies under the very large urinary bladder (u. b.). Primary and secondary sperm ducts are seen, and on the right side three tubules are forming. The testis is composed entirely of stroma. g = gut. $\times 33$.
- Fig. 9. 18 mm. embryo, male. Transverse section of anterior part of the gonadal fold. The gonad is very small and contains small groups of crowded primordial germ cells, and a small blood vessel. The Wolffian duct above it is cut three times. $\times 143$.
- Fig. 10. 18 mm. embryo, female. Transverse section of the ovary in the posterior region showing beginning formation of the ovarian cavity. The mid line of the body is towards the left. The space within the ovary is the lumen of a blood vessel. $\times 143$.
- Fig. 11. Same embryo. Section of the ovary anterior to last figure. Ovarian folds are approaching. $\times 143$.
- Fig. 12. Same embryo. Section through the anterior part of the ovary. The ovarian cavity is complete. Numerous germ cells are shown in all three figures (10-12). $\times 143$.
- Fig. 13. 23 mm. embryo, female. Transverse section of the ovary showing germ cells in all stages of enlargement to form oocytes. The lateral wall (left side) of the ovarian cavity is very thin and has collapsed. $\times 143$.





7



9



10



11



12



13



8

MORE PRIMITIVE MOSS-MITES OF NORTH CAROLINA

By ARTHUR PAUL JACOT

PLATE 20

The species of Oribatidae here described are all Hypochthoniinae as delimited in my earlier paper (2). The species of Brachychthonius are the smallest of the Oribatidae and colorless to orange. Although common in all litters they are usually overlooked because of their minute size and pale coloring. The species here treated fall into two groups—the smooth and the “sculptured.” In the smooth species the bristles usually occupy the same relative positions. The specific differences are therefore limited to the modifications of the bristles themselves and of the pseudostigmatic organs. In the sculptured species the same remarks obtain except that the sculpturing forms an additional and a conspicuous specific character. Breadth of abdomen is a much more constant measurement than length.

KEY TO GENERA OF EAST AMERICAN HYPOCHTHONIINAE

1. Abdomen with only two distinct transverse plates... .. *Hypochthonius*
1. Abdomen with three transverse plates 2
2. Anterior transverse suture interrupted or indistinct in middle... *Eniochthonius*
2. Anterior transverse suture quite distinct throughout 3
3. Size small (less than 0.2 mm. long); sides without lateral plates
Brachychthonius
3. Size larger (0.25 mm. or more); sides with four small lateral plates
Eobrachychthonius

***Brachychthonius perpusillus* (1, p. 220, fig. 41)**

Figure 1

Diagnostic characters: Bristles of dorsal face of medium length, somewhat acicular-foliaceous, the sides flaring upward and laterad from the “midrib;” pseudostigmatic organs with short head bearing about five bristles longitudinally in several rows.

Description: Color pale; size medium (for the genus): length 0.17 mm., breadth 0.096 mm.; rostrum broad, rostral bristles inserted fairly close to anterior edge, projecting well beyond end of rostrum; inter-lamellar bristles inserted rather far forward, length of rostral bristles

distant from rostrals, broader than most of the bristles, deeply longitudinally folded (see figure to left of numeral 1); exopseudostigmatic bristles with projecting midrib (figure to right of numeral), the sclerotized ring large and poorly defined, no clear-cut dorsolateral (lamellar) ridge; interlamellar bristles erect, inserted quite close to end of pseudostigmata, with more than one longitudinal rib; notogastral bristles all of one pattern; their positions (as in figure 1) quite constant, a2 usually extending posteriad above edge of abdomen, a3 on ventrolateral face, directed laterad; bristles of transverse plate II inserted slightly nearer anterior edge of plate than to posterior edge, especially c2.

Cotypes: Twelve specimens from the F-layer of litter of isolated, forty-year-old short-leaf pine stand among closely grazed *Andropogon* pastures on Asheville-Brevard road twelve miles southwest of Asheville, N. Car.; taken October 15th, 1934, slide 34F10.2B1.

***Brachychthonius latus* sp. nov.**

Differs from *B. perpusillus* in that all bristles of dorsal aspect are longer, more slender, and curved; interlamellar bristles directed forward; dorsal plate bristles of second and third transverse rows overlap insertion of the bristle behind them. Size and color seem to be the same though *B. latus* is slightly broader, especially at the shoulders. The bristles are inserted in exactly the same places.

The following are identical with specimens from Regensburg, Germany: sixty-eight specimens from upper part of F-layer of litter of thirty-year-old white pine plantation, Biltmore estate, eight miles from Asheville on Brevard road; taken October 8, 1934, slide 34F9-2h.

***Brachychthonius fimbriatus* sp. nov.**

Figures 2 to 4

Similar to *B. perpusillus* but pseudostigmatic organs long, distal end curved backward and downward, head furnished with ten to twelve bristles linearly; exopseudostigmatic rings well developed, angular, the bristle appearing coarsely triquetrous to quinquetrous; lamellar and interlamellar bristles erect, and, like the notogastral bristles, expanded laterally as very thin wings which are more or less transversely wrinkled and fimbriate (figures 5 and 6), membrane narrowing rapidly to distal end. This membrane is so fine as to be very difficult to discern even under oil immersion, except where the transverse wrinkles are seen along their faces when enough density or shadow is formed to become more visible. The midrib of the bristle is always quite distinct, giving

the animal the appearance of *B. latus*. The fimbriation is more visible in dirty specimens, and on shoulder bristles and bristles of posterior edge of abdomen. Color usually pale. Size large (for the genus): length 0.197 mm., breadth 0.1 mm.

Cotypes: Thirty-seven specimens from sod of *Andropogon bald* (Glen Bald), Bent Creek Experimental Forest, ten miles southwest of Asheville; taken April 17th, 1935, slides 34F31-2, -3, and -6.

***Brachychthonius bifurcatus* sp. nov.**

Figures 5 and 6

Differs from *B. perpusillus* in that all bristles of dorsal aspect are shorter, more slender, and stiff; rostrum distinctly set off; ridges on sides of cephaloprothorax better developed (figure 5); pseudostigmata with funnel straight and stout; pseudostigmatic organs with head more compact so that no distinct bristles are evident, distal end deeply notched (figures 5 and 6); bristles of middle segment inserted *posterior* to transverse center of plate; color quite a distinct orange-yellow, the bristles colorless; size of females: length 0.184 mm., breadth 0.1 mm.

Cotypes: Fifteen specimens from litter of twelve-year-old *Andropogon* pasture with scattered two to three year old pines, Cook property, Brevard road nine miles from Asheville; taken February 19th, 1934, slide 34F26-1.

***Brachychthonius italicus spiciger* comb. nov.**

Figure 7

The first sculptured *Brachychthonius* to be described is *B. brevis italicus* (1, p. 220). As *B. italicus* is not a subspecies of *B. brevis* Michael (non Berlese = *B. berlesei* Willmann (7, p. 160) it stands as a full species. Thus *B. brevis expositus* (1, p. 220) is a short bristled smooth species (or subspecies) closely related to *B. brevis* Michael (4). *Brachychthonius brevis spiciger* of Lake City, Florida is described (1, p. 220) as having a longer, slenderly fusiform pseudostigmatic organ and somewhat different sculpturing than *B. berlesei* which I recognize as a distinct species. The common sculptured species of the southern Appalachians which I regard as *B. spiciger* resembles much more closely *B. italicus* in sculpturing. I am therefore calling it *B. italicus spiciger*.

Diagnostic characters: Bristles of dorsal aspect quite short and fine; pseudostigmatic organ head long, slender, inconspicuously roughened (*spiciger* = bearer of wheat heads); sculpturing of angular designs (figure 7); posterior edge of abdomen broadly rounded, not angled.

Description: Color pale; size medium: length 0.17 mm., breadth 0.09 mm.; cephaloprothorax somewhat angular, with well developed ridges; rostrum distinct, the bristles prominent, longer than the other body bristles; lamellar bristles plicate, exopseudostigmatic ring conspicuous, angled; pseudostigmata with slender cells; vertex with a cluster of three pairs of more or less rectangular areas formed by slender ridges; bristles a2, a3, and b3 quite close to each other, bristles c1 and c2 nearer posterior than anterior edge of plate.

Material described: Forty-seven specimens from L-layer of litter of dogwood of thirty-year-old-field woodland, Bent Creek Experimental Forest; taken September 20th, 1934, slide 34F4.1-2.

***Brachychthonius rostratus* sp. nov.**

Figure 8

Diagnostic characters: Bristles of dorsal aspect very short, fine; pseudostigmatic organ head slender with four or five bristles in linear series; sculpturing comprised of clusters of ovate spots of granules (much resembling oil globules on the surface of bouillon) (figure 8, where the granules are indicated in a few of the spots); posterior end of abdomen deeply and broadly emarginate.

Description: Color pale honey yellow; size smallish: length 0.168 mm., breadth 0.0877 mm.; cephaloprothorax, especially anterior end, quite angular; rostrum distinct, bristles not discernible; ridges well developed, a secondary ridge mesad of the lateral; vertex with a row of four rectangular areas formed by slender ridges; bristles a2, a3, and b3 not discernible; bristles b2 inserted quite laterad; bristles c1 and c2 nearer anterior than posterior edge of plate.

Cotypes: Eight specimens from sod of *Andropogon bald*, top of Shut-in-Ridge, Bent Creek Experimental Forest; taken May 8th, 1935, slide 34F34-19.

Brachychthonius brevis Michael (4) is a smooth species with slightly serrate (see figure) rostral bristles, interlamellar bristles somewhat spine-like, abdomen almost parallel-sided, bristles of dorsal plates thick. If it were not for the serrulate rostral bristles, I would regard *B. perpusillus* as possibly synonymous. *Brachychthonius perpusillus* Berlese (1, p. 220) is very similar as to caliber and length of dorsal bristles, and length of pseudostigmatic organs. *Brachychthonius brevis* Willmann (7) has shorter and finer bristles and in that respect approaches *B. bifurcatus*. *Brachychthonius perpusillus* Willmann (7) has bristles

of dorsal face of abdomen resembling those of *B. latus*. All these European species are so poorly described and figured (except Michael's) as to make it impossible to make detailed comparisons. Henceforth special attention should be paid to character (relative length, breadth, and shape) of bristles, especially the rostral, exopseudostigmatic, interlamellar, and dorsal plate, as well as pseudostigmatic organs. The exact location of bristles of the middle transverse plate is important. Bristles b2 tend to migrate toward lateral edge of abdomen.

***Eniochthonius pallidulus* (3)**

Specimens from the eastern states south to the southern Appalachians are identical with specimens from Regensburg, Germany. I have them from cove litter of the Bent Creek Experimental Forest.

***Hypochthonius gracilis* sp. nov.**

Figure 9

Diagnostic characters: Body slender; interlamellar bristles erect, clavate; notogastral bristles twenty-four, long, overlapping, tapering, slightly curved.

Description: Color pale; size rather small: length 0.31 mm., breadth 0.14 mm.; rostrum fairly broad, edge smooth; rostral bristles rather short, not extending much beyond rostrum; lamellar bristles medium long, inserted in longitudinal middle of cephaloprothorax, directed backward; sides of cephaloprothorax with a short ridge; exopseudostigmatic bristles rather short; pseudostigmata with four cells, the organ with about ten cilia; no depressed angle at anterolateral corners of notogaster, nor at posterolateral areas; ventral plate with two rather short bristles each side; anal covers with two insertions; paranal plates with three insertions; genital covers transversely divided, each half with three mesal and two lateral insertions (figure 9); parasterna with bristles as in figure 9.

Cotypes: Twelve specimens from fallen, damp leaves, horticultural grounds, Gainesville, Fla.; taken February 29th, 1928, by Edgar F. Grossman, slides G30H1 to -H3.

This species also occurs in the Bent Creek Experimental Forest area, especially in litter of old woodlands. Five specimens from leaf litter, Rocky Cove, Bent Creek Experimental Forest; taken September 8th, 1934, slides 34F1-1 and -18. Four specimens from pine-oak woodland, section 18 of same Forest; taken October 1st, 1934, slide 34F7-3.

Hypochthonius rufulus carolinicus (2)

In this subspecies the pseudostigmatic organs have fifteen or more pectinations; the bristles of dorsal plate are closely barbed, long, curved, tapering. It is represented by small numbers in square foot samples of woodland litter.

Hypochthonius luteus (7, p. 24, pl. 16, figs. 16-19) (8, p. 21)

Specimens from the Bent Creek Experimental Forest area are identical with those from Regensburg in Bavaria. The pseudostigmatic organs have ten to thirteen pectinations; the bristles of dorsal plates are shorter than in *H. rufulus*, stiff, rough, and blunt.

Two specimens from sod of *Andropogon bald*, top of Shut-in-Ridge, Bent Creek Experimental Forest; taken May 8th, 1935, slide 34F34-20. One specimen from litter of old field grown to pines, Stradley Hill, nine miles southwest of Asheville on Brevard road; taken October 30th, 1934, slide 34F15.201.

U. S. FOREST SERVICE,
ASHEVILLE, N. C.

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PLATE 20

Brachychthonius perpusillus sp. nov.

Fig. 1. Dorsal aspect, legs omitted; $\times 529$. Details of bristles free hand.

Brachychthonius fimbriatus sp. nov.

Fig. 2. Dorsal aspect of cephaloprothorax, and an additional pseudostigmatic organ; $\times 529$.

Fig. 3. Posterior edge of abdomen with two of the bristles; $\times 529$.

Fig. 4. A mesal bristle of abdomen; free hand.

Brachychthonius bifurcatus sp. nov.

Fig. 5. Dorsal aspect of cephaloprothorax, legs omitted; $\times 529$.

Fig. 6. A pseudostigmatic organ; free hand.

Brachychthonius italicus spiciger (1)

Fig. 7. Dorsal aspect, legs omitted; $\times 529$.

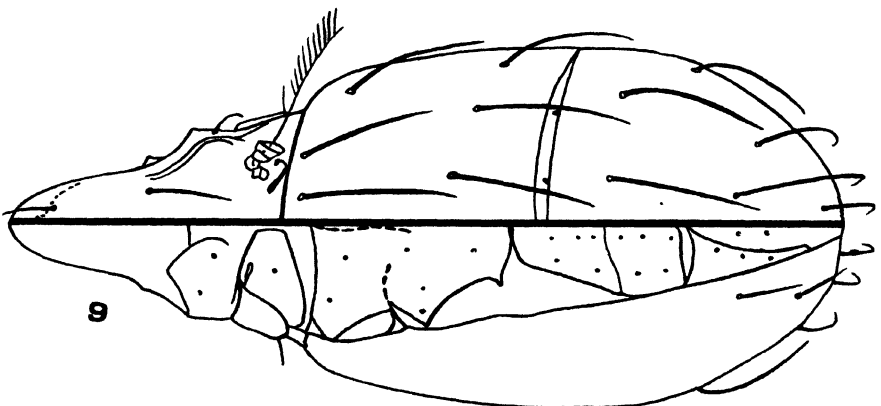
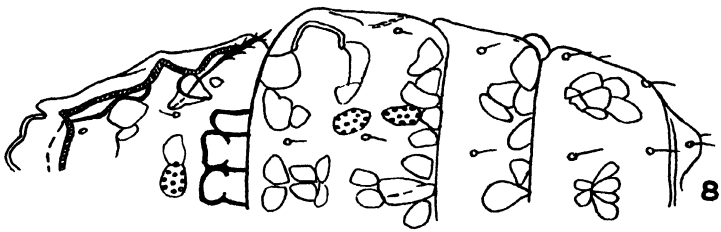
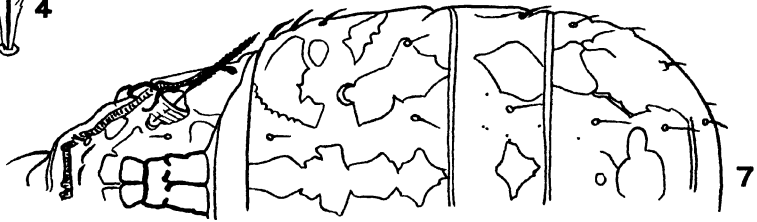
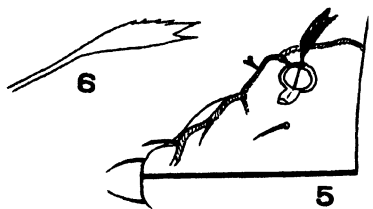
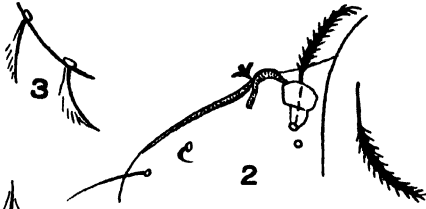
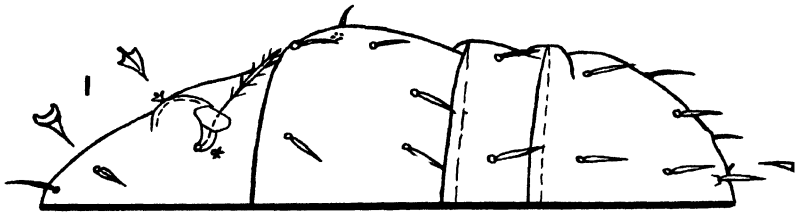
Brachychthonius rostratus sp. nov.

Fig. 8. Dorsal aspect, legs omitted; $\times 529$.

Hypochthonius gracilis sp. nov.

Fig. 9. Dorso/ventral aspects, legs and mouth parts omitted; $\times 329$.

PLATE 20



ORIBATA AND BELBA FROM FLORIDA (ACARINA, ORIBATINAE)

By J. W. WILSON

PLATE 21

The material described in this paper was collected by Mr. Edward F. Grossman and a few others of the Experiment Station staff during the spring and early summer months of 1928. The collections were all made by collecting leaves and debris which were placed in Berlese funnels to trap the mites. The specimens were then mounted on slides in balsam. Jacot (1933 and 1935) gives a map of Florida showing the location of most of the points at which collections were made and the field notes of Grossman relating to these collections. This collection made by Grossman covers the whole state and represents a wide variety of ecological conditions. In this paper the number preceding the dash on each slide represents the lot number in Grossman's field notes. In the notes on places where collections were made, the year (1928) is not repeated and where no collector is named the specimens were collected by Grossman.

The author wishes to acknowledge Professor J. R. Watson's assistance in securing the collection and his continued interest in the study of this material. To Dr. Arthur P. Jacot the author is indebted for many helpful suggestions and much encouragement in the execution of this work. Dr. H. E. Ewing of the National Museum has very kindly loaned specimens for purposes of comparison.

In this paper the author has followed Jacot (1929 and 1934) in his definitions and restrictions of the tribe *Oribatinae* and the genera included therein. In these papers Jacot presents evidence indicating that *Oribata* Lat. belongs to the apterogastinae group of Acarina instead of to the pterogastinae where it was placed for a long time even by such careful workers as Michael. It is also shown that the genus *Damaeus* Michael is probably a synonym of *Oribata*.

Figures of *Oribata grossmani* sp. n. were sent to Professor Nathan Banks of the Museum of Comparative Zoology which he compared with his type of *O. angustipes* Banks. Professor Banks replied as follows: "Your figures do not represent *O. angustipes* but some allied species.

The long bristles on hind legs do not appear broken, and are alike on both hind legs, about as long as the long one near tip of tibia. However, there is a more important difference; the hind tarsi are much longer than your figure, the part beyond the swelling being fully twice as long as your figure. The tibia is fully as long as your figure. The front tarsus is also longer but not so much longer than your figure."

***Oribata grossmani* sp. n.**

Figures 1, 5 and 8

DIAGNOSIS

Cephalothorax with longitudinal ridges each side joining a short curved transverse ridge in front of each pseudostigmatum (fig. 5), tectopodia I and II prominent. Dorsal part of body and legs thickly studded with short tubercles. Notogaster bearing on each side of anterior margin a stout forewaist spine which reaches almost to the base of the pseudostigmata. Legs very long and slender, bearing a few very long bristles.

DESCRIPTION

Rostrum rather long, tapering to a point and bearing above two pairs of long rostral hairs, one at sides, and one on the dorsum. Cephalothorax suddenly wider in front of coxae I, thickly studded with short tubercles, with ridges as shown in figure 5; exopseudostigmatic bristles short, fine, interlamellar bristles finely serrated, curved, extending back to the anterior margin of the notogaster; pseudostigmata funnel-shaped; pseudostigmatic organ very long, tapering to a fine thread-like end, finely serrated along basal portion (fig. 8); tectopodia I and II quite prominent, easily seen from both dorsal and ventral view.

Abdomen round, very convex with a wide lateral band between notogaster and ventral plate, notogaster not covering anterior lateral angles of abdomen, surface of notogaster covered with short tubercles, two rows of 8 curved, short, stout, finely serrated bristles near median line, three short, curved bristles near posterior margin. The anteriormost bristles are near the anterior margin and closer to the median line than the remaining bristles. On the anterior part of the notogaster there are two stout, curved spines (forewaist spines) reaching almost to the base of the pseudostigmata.

Ventral plate projecting as a shelf ventrad of leg II to leg IV, this shelf with well developed projections in front of leg III and in front of

and behind leg IV. Anal aperture near posterior edge of ventral plate, genital aperture close to anal aperture. Sides of anal covers almost straight, curving posteriorly, anterior edge deeply emarginate. Preanal and postanal bristles stout and short, close to the anal aperture. Preanal bristles located slightly ventrad of anterior margin of anal covers, lateral postanals at center of covers and the mesal postanal pair near posterior edge of anal cover; anal cover bristles short, nearer lateral edge of covers than mesal edge, the anterior near anterolateral angle, the posterior near center of covers and in line with lateral postanal bristle; paramesal bristles slightly anteriad of posterior angle of genital aperture. Genital aperture broadly oval; four genital cover bristles, the first two on middle line of cover, the posterior two nearer lateral edge, genito-thoracic suture not visible. Parasterna III and IV each bear three long fine bristles arranged nearly in a straight line, parasterna I and II each with a single bristle located as indicated in figure 5. Camerostome large, elongate oval, labial bristle inserted near the lateral margin.

Legs terminated by a single slightly curved hook, armed with short and long bristles. Leg IV the longest, leg II the shortest, legs very long and slender.

Leg I (fig. 1) longer than leg II. Tarsi fairly broad near proximal end, dorsal face with four bristles; proximal inserted at the broadest part of the segment, bristle 2 inserted far from 1 on slender distal part of tarsus, bristle 3 about one-third the distance from 2 as 2 is from 1, bristle 4 about equal distance between 3 and distal end of tarsus, bristles 2, 3, and 4 fine and short, only plainly visible under high magnification: ventral face with six bristles, bristle 1 more proximal than 1 of dorsal face, bristle 2 inserted at the point where the tarsus becomes narrower, bristles 3, 5, and 6 inserted opposite 2, 3, and 4 of dorsal face, bristle 4 equidistant between 3 and 5, bristles 3, 4, 5, and 6 similar to 2, 3, and 4 of the dorsal face, a lateral bristle inserted near dorsal bristle 1, borne on a tubercle, another inserted a little more distad and on the lateral median line, a mesal bristle inserted slightly distad of ventral bristle 1, another inserted on the median line and slightly more proximal of dorsal bristle 1, all bristles on tarsi smooth. Tibiae only a little shorter than tarsi, slightly swollen at distal end; two bristles on dorsal surface, a finely serrated major bristle midway between proximal and distal ends of tibiae, a smooth minor bristle inserted at the distal end; four smooth bristles on ventral surface spaced equidistant apart; two bristles on lateral surface; one finely serrated bristle inserted near ven-

tral bristle 3, one smooth bristle borne on a tubercle near dorsal surface and distal end of tibiae, no bristles on mesal surface. Genuals one-third length of tibiae, with two finely serrated bristles on dorsal face inserted very close together, one long, finely serrated bristle inserted on lateral face near ventral face and more proximad than the two dorsal bristles, one finely serrated, short bristle inserted on ventral face midway between proximal and distal ends, one long finely serrated bristle on mesal surface inserted on same transverse plane as lateral bristle but nearer dorsal surface (not shown in fig. 1). Femora long, slightly curved and enlarged at distal end; dorsal face with three finely serrated bristles, one longer and stouter than the other two, inserted near proximal end of segment, bristles 2 and 3 inserted near the widest part of segment; ventral face with two finely serrated bristles, bristle 1 inserted two-thirds distance from proximal end of segment, bristle 2 inserted near beginning of enlargement; a long finely serrated bristle inserted on lateral face on same transverse plane as bristle 2 on dorsal face, a finely serrated short bristle inserted on mesal face on the same plane as bristle 2 of the ventral face. Coxae small, cup-shaped, a single smooth bristle inserted at distal end.

Leg II (fig. 1) similar to leg I but each segment proportionally shorter. Tarsi with two additional bristles on dorsal face, no bristle rising from a tubercle on lateral face, two additional bristles on ventral face, two mesal bristles, lateral bristle finely serrated, all the other bristles smooth. Tibiae with one bristle on dorsal face, a long, stout finely serrated bristle on both lateral and mesal faces, these two bristles opposite, three bristles on ventral face. Genua with one bristle on each face, all finely serrated. Femora with two bristles on dorsal face, two on lateral face, one on ventral face and two on mesal face, all finely serrated. Coxae same size as coxae of leg I, with a long, curved, smooth bristle inserted on ventral face near distal end.

Leg III longer than leg II but not as long as leg IV. Tarsi shorter than tarsi IV, dorsal face with three bristles, bristle 1 inserted at widest part of segment, finely serrated, bristle 2 and 3 at distal end of tarsi, lateral face with one long, finely serrated bristle inserted slightly distad of dorsal bristle 1, ventral face with five short, smooth bristles; mesal face with two short smooth bristles, one inserted near proximal end and one slightly distad of the bristle on lateral face. Tibiae shorter and more robust than tibiae IV, one finely serrated bristle on dorsal face, two smooth bristles on lateral face, three on ventral face, one on mesal face. Genuals shorter than genuals IV, one long bristle on dorsal

face, one on lateral face, none on ventral face, and two on mesal face, all finely serrated. Femora stouter and shorter than femora IV; three short finely serrated bristles on dorsal face, one long, smooth bristle on lateral face, inserted at widest part of segment, no bristles on ventral face; one on mesal face, inserted on same transverse plane as bristle 2 of dorsal face. Coxae stouter than coxae IV and with two finely serrated bristles on dorsal face.

Leg IV longest and slenderest of all the legs. Tarsi with five smooth, short bristles on dorsal face, one long, finely serrated bristle on lateral face, four short, smooth bristles on ventral face, and no bristles on mesal face. Tibiae very long and slender, only slightly swollen at distal end, with one long, finely serrated bristle on dorsal face, one short finely serrated bristle on lateral face, no bristles on ventral face, five short, smooth bristles on mesal face. Genuals slender with one short finely serrated bristle on dorsal face; one very long (extending beyond distal end of tibiae), smooth bristle on lateral face, inserted near distal end; no bristles on ventral face; one short finely serrated bristle on mesal face. Femora long, slender, swollen at distal end, with three finely serrated bristles on dorsal face; a very long, smooth bristle on lateral face (not as long as bristle on genuals, but extending half length of tibiae); no bristles on ventral or mesal face. Coxae gourd-shaped, with a single short finely serrated bristle on dorsal face.

Color, reddish tan.

Dimensions of five specimens given in microns are as follows:

	<i>Greatest</i>	<i>Average</i>	<i>Smallest</i>
Total length of body.....	666	632	599
Length of notogastral plate.....	432	397	346
Breadth of notogastral plate.....	412	384	366
Interlamellar bristle span.....	80	77	66
Camerostome to genital aperture.....	166	146	133
Length of genital aperture.....	133	113	100
Breadth of genital aperture.....	133	116	100
Genital aperture to anal aperture.....	39	38	26
Length of anal aperture.....	120	94	66
Breadth of anal aperture.....	93	78	53

Specimens studied:

22 Specimens from Villa Tasso, Choctauhatchee Bay, Fla., from fallen leaves of oak, Magnolia, and hickory, May 18, R. W. Blacklock. Slide Nos. 101-D31, 101-D35, 101-D38, 101-D30, 101-D39, 101-D41, 101-D51. One specimen from *Asparagus plumosus* Pierson, May 18, Erdman West, Slide no. 100-D47. Two specimens from fallen water oak leaves, near

Mulberry, May 17, Erdman West, Slide Nos. 103-D48 and 103-D50. Four specimens from fallen leaves, Gainesville, April 20, Slides Nos. 73-D29, 73-D43, 73-D44, 73-D52. One specimen from dry leaves on ground, Bonita Springs, May 4, J. R. Watson, Slide No. 95-D37. Four specimens from fallen hickory leaves, Pinkoson Springs near Gainesville, March 14, Slide Nos. 33-D40, 33-D42, 33-D46. Seven specimens from fallen oak leaves, Devil's Mill-Hopper near Gainesville, April 4, Slide No. 75-D32. Two specimens from dry leaves, Gainesville, May 10, H. E. Bratley, Slide Nos. 90-D33, 90-D34. One specimen from fallen elder, Magnolia, oak leaves, Gainesville, February 29, Slide No. 29-D36. One specimen, leaves on beach, North Beach, St. Augustine, April 1, Slide no. 63-D45.

Holotype, Slide No. 101-D39.

***Belba globifer florida* subsp. n.**

Figure 6

DIAGNOSIS

Differing from *B. globifer* in that the pseudostigmatic organ is smooth instead of slightly pectinate and that the spines projecting from the front of the abdomen are straight instead of curved.

DESCRIPTION

Cephalothorax broad and plain, without ridges, furrows or bands; rostrum rather short and rounded at anterior end, rostral bristles stout and curved inward; interlamellar bristles stout and curved outward; exopseudostigmatic bristle stout and curved forward.

Abdomen almost perfectly round; notogaster smooth, high, regularly arched; margin smooth; bristles long, stout, only slightly curved, there are eight bristles in each row placed near the center of each side of the notogaster; three short bristles near posterior margin, from the waist there are two stout straight, cone-shaped spines whose tip is some distance from the base of the interlamellar bristles.

Ventral plate well developed, round, not encroached on by the notogaster; triangular tooth projecting from margin between legs III and IV; anal aperture close to posterior margin, broadly rounded posteriorly, sides tapering slightly, anterior edges slightly rounded; anal covers convex with three bristles located as shown in figure 7; preanal bristle well toward lateral margin of ventral plate and on same transverse plane as anterior anal cover bristle; mesal postanal bristle near pos-

terior edge of anal aperture; two bristles posterior to transverse plane tangent to anterior margin of genital aperture, one near lateral margin and one near center of ventral plate; three bristles on transverse line anterior to genital aperture. Genital aperture almost round, rather large with five fine bristles on genital covers in positions indicated in figure 6; genito-thoracic suture not visible from ventral view. Sternum with two bristles (figure 6), parasternum II with one bristle.

Legs terminated by a single well developed hook, and armed with rather stout smooth bristles, legs IV the longest.

Legs I much longer than legs II, coxae without bristles. Femora with long slender proximal portion, large swollen portion distally, two stout bristles on dorsal face, no bristles on external side, two bristles on ventral face, one near center and proximal end of swollen portion, the other slightly distad of first and toward inner side, inner side with one bristle on same plane as the more proximal bristle on dorsal face. Genuals short, with two bristles on dorsal face, one on inner side, one near center and proximal end of swollen portion, and one on outer side. Tibiae two-thirds as broad as long, major bristle on outer side twice as long as the segment, a short fine bristle slightly distad on the dorsal face with a stout long bristle near center of dorsal face, a stout long bristle on same plane on the inner side, two bristles on about same plane on ventral face, one stout and long near center and one short and fine near inner side. Tarsi with short proximal neck, dorsal face with three bristles, outer side bearing short major bristle and two others on slender distal portion of segment, no bristles visible on ventral face, inner side with five bristles arranged as shown in figure 6.

Legs II with each segment relatively shorter than in leg I, longer than leg III. Coxae similar to those of legs I. Femora with long slender proximal portion and large swollen distal portion, two bristles on dorsal face, one on outer side, two on ventral face, one at proximal end of slender portion, and one near center of proximal end of swollen portion, one on inner side (fig. 6). Genuals slightly less than half as broad as long, one bristle on dorsal, outer and inner faces, none on ventral face. Tibiae with two bristles on dorsal face and one on outer side. Tarsi with five hairs on each of the inner and outer sides, three shorter and finer hairs on dorsal face, and three on ventral face.

Legs III shorter than legs II. Coxae gourd-shaped with a sharp tooth-like projection on the internal face, internal part of coxae rounded except for the tooth-like projection, external part neck-like, irregularly swollen distal portion, one short fine bristle on dorsal face, and one long

stout bristle on outer side. Femora with two stout, long bristles on dorsal face of swollen portion, one stout bristle on outer side. Genuals short and small with two bristles on dorsal face, one on outer side and one on inner side. Tibiae nearly half as long, one bristle on dorsal face, one on outer side, two on ventral face. Tarsi with five bristles on outer side, one on ventral side near distal end, four on inner side, none on dorsal face.

Legs IV with genuals reaching to end of abdomen. Coxae with inner rounded portion, long curved slender portion swelling into rounded squash-shaped distal portion with one stout bristle on ventral face. Femora half as broad as long with two bristles on dorsal face and two on ventral face. Genuals short, slightly enlarged at distal end, one bristle on dorsal, outer and ventral sides. Tibiae longer, not quite half as broad as long, one bristle on dorsal face, one on outer side and two on ventral face.

Dimensions of five specimens measured in microns are as follows:

	<i>Greatest</i>	<i>Average</i>	<i>Smallest</i>
Total length of body.....	413	391	366
Length notogastral plate.....	279	241	213
Breadth notogastral plate.....	246	207	186
Interlamellar bristle span.....	86	76	60
Camerostome to genital aperture.....	100	85	80
Length of genital aperture.....	73	70	60
Breadth of genital aperture.....	66	64	60
Genital aperture to anal aperture.....	33	23	20
Length of anal aperture.....	86	74	66
Breadth of anal aperture.....	66	64	53

Specimens studied:

One specimen from leaves of sweetgum, near Micanopy, April 17, Slide no. 74-D24. Cotypes: One specimen from hickory leaves, Pinkoson Springs, March 4, Slide number 33-D22. Two specimens, Bay Shore debris, Vero Beach, Erdman West, April 6, Slides nos. 67-D23 and 67-D19. One specimen, dry oak leaves, Horticulture grounds, Gainesville, H. E. Bratley, April 27, Slide no. 84-D27. One specimen, dry leaves, Gainesville, April 2, Slide no. 75-D6. One specimen, elder, Magnolia, and oak leaves, Horticulture grounds, Gainesville, February 29, Slide no. 29-D21; two specimens from pine and oak leaves, Wellborn, May 30, Slide nos. 115-D1 and 115-D11.

Belba michaeli (Ewing) 1909, p. 129

Under date of February 1, 1936, Dr. H. E. Ewing wrote in reply to a request for the loan of a type specimen of *Damaeus michaeli* that "All

of the original types of this species have been lost or misplaced, but I have an autotype specimen which was collected the same year that the species was described."

Upon studying this autotype specimen sent by Dr. Ewing it was found that the illustration of *D. michaeli* did not agree with the description in the following particulars: the description calls for pectinate and flagelliform pseudostigmatic organs while the illustration figures barbed and rod-like pseudostigmatic organs. Thus the description is based on two species. This is borne out by the two habitats mentioned in the description: "In moss and under bark of logs." I am therefore restricting *D. michaeli* to the species with the pectinate, flagelliform pseudostigmatic organs, notogaster and leg segments bearing pectinate bristles; anal and genital covers separated by about one-half their length: and under *Belba jacoti* sp. n. the related species described below.

***Belba jacoti* sp. n.**

Figures 2, 3, 4

DIAGNOSIS

Pseudostigmatic organ slightly shorter than cephalothorax, straight, not flagelliform, barbed. Notogaster with 18 stout, slightly curved smooth spines; surface of notogaster smooth. Legs moniliform, each joint with one or more stout curved and barbed bristles. Abdomen globular. Anal and genital apertures close together.

DESCRIPTION

Cephalothorax, as seen from above, pyriform, broad, sides tapering gradually to the blunt, conical rostrum; tectopodia invisible both from the dorsal and lateral views; surface smooth; two pairs rostral bristles, medium long; interlamellar bristles stout, of medium length; exopseudostigmatic bristles short and stout; pseudostigmata cup-shaped; pseudostigmatic organ rod-like, slightly expanded distally and barbed, then tapering to a fine point.

Abdomen high, circular, without forewaist spines; notogaster smooth, bearing 18 stout slightly curved bristles which in the adult are smooth, notogaster frequently carrying the cast skins of previous molts, the spines of the immature stages being barbed. The posteriormost bristles are well down on the notogaster; three short bristles each side near posterior margins.

Ventral plate with posterior edge slightly undulating. Anal aperture close to posterior edge of ventral plate, broadest posteriorly, tapering

slightly toward the anterior end, anal covers each with two long fine bristles, one near the anterior margin and one near the center. The postanal bristles long and fine; mesal postanal bristles between posterior margin of anal aperture and posterior edge of ventral plate; lateral postanal bristles near the anal aperture at the posterior angle, preanal bristles on the same transverse plane as the center anal cover bristle, six paramesal bristles located on a curving line from posterior of genital aperture to lateral edge of ventral plate, one bristle near center of ventral plate between coxa IV and genital aperture, four long bristles on a straight line from anterior margin of coxa IV to center of genital aperture, two long, fine bristles on a line anterior of genital aperture, one long fine bristle near suture between cephalothorax and ventral plate (fig. 4). Genital aperture oval, genital covers with five well developed cover bristles placed as shown in figure 4, genito-thoracic suture not visible, three bristles on parasternum I arranged as in figure 4, the one near the lateral margin much longer than the other two, one bristle on parasternum II near coxae II (fig. 6).

Legs moderate in length, moniliform, each terminated by a well-developed, strongly curved hook, armed with one or more stout, curved, barbed bristles on all segments except the coxae. Legs IV the longest, legs III the shortest.

Legs I (fig. 6) longer than legs II. Coxae curved and slender at body, slightly larger at distal end with one bristle on dorsal surface. Femora with long spindle-shaped portion and much enlarged distal portion, one strong, barbed dorsal bristle, three outer lateral bristles, one about the center of the spindle portion and two on the enlarged portion, one ventral bristle near the distal end and one inner lateral bristle. Genuals squash-shaped with three stout barbed bristles, one dorsal, one outer lateral and one inner lateral. Tibiae shorter and larger than genuals, with five bristles, two dorsal, the major bristle on the outer lateral side, one ventral and one inner lateral bristle. Tarsi with enlarged portion near proximal end, with three bristles on dorsal face, three on outer lateral side, two on ventral face, and three on inner lateral side.

Legs II similar to legs I, but each segment slightly shorter. Coxae with one bristle on dorsal surface, femora with one dorsal bristle, three outer lateral, one ventral, and two inner lateral bristles. Genuals with three bristles, one dorsal, one outer lateral, and one inner lateral. Tibiae with one dorsal, one outer lateral, two ventral, and one inner lateral bristle. Tarsi with two dorsal bristles, four outer lateral bristles, four ventral bristles, and three bristles on inner lateral side.

Legs III shorter than legs II. Coxae greatly enlarged with two bris-

tles, one on outer lateral side and one on ventral face. Femora with one dorsal bristle, three outer lateral bristles, and two ventral bristles. Genuals with one dorsal, one outer lateral and one ventral bristle. Tibiae with one dorsal, one outer lateral and two inner lateral bristles. Tarsi with five outer lateral bristles, one on the enlarged portion, four ventral bristles, two on enlarged portion and two on shaft, three inner lateral bristles, one on the enlarged proximal portion, and two on the shaft, two dorsal bristles on the shaft.

Legs IV the longest. Coxae with a long cylindrical proximal portion and enlarged distal portion bearing one outer lateral bristle. Femora with one dorsal, two outer lateral, and one ventral bristle. Genuals with one dorsal, one outer lateral, and one ventral bristle. Tibiae with one dorsal, two outer lateral, and one ventral bristle. Tarsi with three dorsal bristles, one on the enlarged proximal portion, two on the shaft, two ventral bristles on the enlarged portion, and one on the shaft, and five outer lateral bristles.

Dimensions of three specimens given in microns are as follows:

	<i>Greatest</i>	<i>Average</i>	<i>Smallest</i>
Total length of body.....	479	441	413
Length notogastral plate.....	313	297	266
Breadth notogastral plate.....	266	266	266
Interlamellar bristle span.....	113	93	73
Camerostome to genital aperture.....	113	108	100
Length of genital aperture.....	73	67	66
Breadth of genital aperture.....	73	73	73
Genital aperture to anal aperture.....	27	24	20
Length of anal aperture.....	93	86	80
Breadth of anal aperture.....	73	70	66

Specimens studied:

Three specimens from oak leaves and moss, Neumans Lake, Gainesville, March 25, Slide nos. 55-D14, -D17, -D18; three specimens from leaves on beach, St. Augustine, April 1, Slide nos. 63-D7, -D25, -D26; one specimen from small leaves of *Tamola littoralis*, St. Augustine, March, Slide no. 34-D.

Holotype, Slide No. 55-D14.

Belba alachua sp. n.

Figure 7

DIAGNOSIS

Body less heavily chitinized than *B. jacoti*. Cephalothorax smooth, pseudostigmatic organ long, flagelliform, smooth. Notogaster with

eight smooth bristles on each side, without forewaist spines. All of the body and leg bristles smooth.

DESCRIPTION

Cephalothorax broad without ridges or other marks. Rostrum rounded with one pair of rostral bristles. Tectopedia not visible. Pseudostigmatic organ very long and slender, ending in fine thread. Exopseudostigmatic and interlamellar bristles close to pseudostigmata, smooth, stout, and slightly curved.

Abdomen ovate, notogaster smooth, bearing 16 smooth, curved bristles arranged as shown in figure 7, and with three short bristles on each side near posterior margin. Ventral plate not encroached upon by notogaster, margin smooth. Anal aperture close to posterior margin of ventral plate; broadest posteriorly, tapering very slightly toward the anterior end; anal covers each with three short, fine cover bristles. The postanal bristles are fine, of medium length; mesal postanal bristles located between anal aperture and posterior margin of ventral plate; lateral postanal located near posterior angle of the anal aperture; preanal nearer the lateral margin of the ventral plate than the anal aperture and posteriad of the anterior margin of anal aperture. Three paramesal bristles on a transverse plane from the center of the genital aperture to anterior margin of coxa IV. Tectopedia IV just visible from the ventral view as a triangular projection between legs III and IV. Three bristles anterior to genital aperture and coxa III. Another bristle just anterior of coxa III. Genital aperture almost round, genital covers each with five short, fine bristles (fig. 7). Two bristles on cephalothoracic sternum; one each on parasterna I and II.

Legs moderate in length, moniliform, each terminated by a single well developed strongly curved hook, armed with one or more, stout, smooth, curved bristles on all segments. Legs IV the longest; legs II and III about equal in length, slightly shorter than legs I.

Legs I (fig. 7) longer than legs II. Coxae slender at the base, suddenly enlarged at juncture with femora, one bristle at distal end. Femora with slender spindle-shaped portion and much enlarged distal portion, one outer lateral bristle near proximal end, three on enlarged distal portion, one bristle on dorsal face, and one on the ventral face. Genuals shorter and smaller in diameter than the femora with one bristle on each of the four faces. Tibiae with two bristles on outer lateral side, major bristle and one other on dorsal face, one bristle each on inner lateral side and ventral face. Tarsi with four outer lateral bristles, two on the

enlarged portion and two on the distal part of the shaft; two dorsal bristles on the enlarged portion; five inner lateral bristles, two on enlarged portion and three on shaft; two ventral bristles on the shaft.

Legs II similar to legs I, slightly shorter. Coxae with a single long, curved bristle at distal end. Femora with two outer lateral bristles, one on the proximal portion and one on the enlarged portion; one dorsal bristle; two inner lateral bristles; no ventral bristles; Genuals with one bristle on each of the four sides. Tibiae larger than genuals with one bristle on each of the four sides. Tarsi with four outer lateral bristles, two on the enlarged proximal portion and two on the shaft; two dorsal bristles on the shaft; six inner lateral bristles, one on the enlarged portion and five on the shaft; two ventral bristles on the shaft.

Legs III (fig. 7) about the same length of legs II. Coxae gourd-shaped with two ventral bristles, one on the proximal slender portion and one on the distal enlarged portion; one outer lateral bristle. Femora with two outer lateral bristles; one dorsal bristle; one inner lateral bristle; one ventral bristle. Genuals with one bristle on each of the four sides. Tibiae with one ventral bristle; one outer lateral bristle; two dorsal bristles, one inner lateral bristle. Tarsi with one ventral bristle near distal end of the shaft; three outer lateral bristles on the shaft and one on the enlarged proximal portion; two dorsal bristles on enlarged portion and one on the shaft; six inner lateral bristles; two on the enlarged portion and four on the shaft.

Legs IV the longest. Coxae gourd-shaped, the proximal slender portion rather long, one ventral bristle at the extreme distal end. Femora with one ventral bristle; two outer lateral bristles; one dorsal bristle. Genuals with one bristle each on the outer lateral side, dorsal side, and inner lateral side. Tibiae with a major bristle similar to the major bristle of legs I developed on the outer lateral face, one dorsal bristle; two inner lateral bristles. Tarsi with one outer long lateral bristle on the proximal portion and two ordinary bristles on the distal portion; two dorsal bristles on the shaft; five inner lateral bristles, two on the enlarged portion and three on the shaft.

Dimensions of two specimens given in microns are as follows:

	<i>Greatest</i>	<i>Smallest</i>
Total length of body	386	386
Length notogastral plate	260	233
Breadth notogastral plate	246	233
Interlamellar bristle span	80	80
Camerostome to genital aperture	106	86
Length of genital aperture	73	66

	Greatest	Smallest
Breadth of genital aperture	73	66
Genital aperture to anal aperture . .	20	20
Length anal aperture.....	80	80
Breadth anal aperture.....	60	60

Specimens studied:

Three specimens from Magnolia, oak and grape leaves, Horticulture grounds, Gainesville, January 21, Slide Nos. 3-D3, and 3-D4.

Holotype, Slide no. 3-D4.

FLORIDA AGRICULTURAL EXPERIMENT STATION,
LEESBURG, FLA.

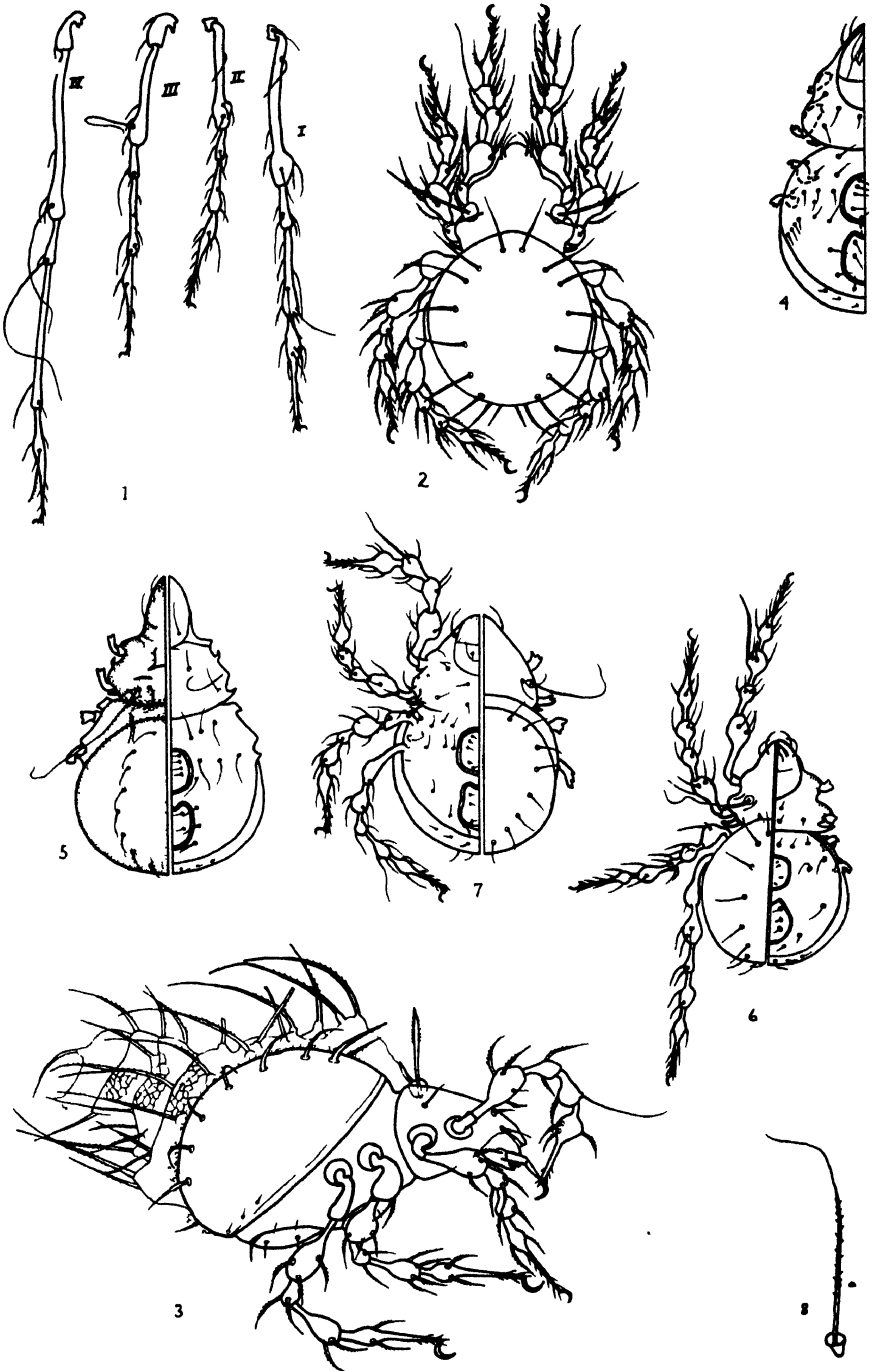
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EXPLANATION OF PLATE 21

- Fig. 1. Legs I, II, III and IV of *O. grossmani* sp. n.
 Fig. 2. Dorsal view of *B. jacoti* sp. n.
 Fig. 3. Side view of *B. jacoti* sp. n.
 Fig. 4. Ventral view of *B. jacoti* sp. n.
 Fig. 5. Dorsal and ventral view of *O. grossmani* sp. n.
 Fig. 6. Dorsal and ventral view of *B. globifer florida* subsp. n.
 Fig. 7. Dorsal and ventral view of *B. alachua* sp. n.
 Fig. 8. Pseudostigmatic organ of *O. grossmani* sp. n.

PLATE 21



THE INCUBATION OF HEN EGGS UNDER INCREASED ATMOSPHERIC PRESSURE

By BERT CUNNINGHAM

A study of this nature must of necessity extend over a long period of time. The period in which one may secure fertile and viable eggs is restricted and the incubation period is relatively long. Furthermore, the usual control and recording instruments do not work satisfactorily under pressure, and new apparatus has to be devised for these purposes. Once a complete set up has been made there are multiplied possibilities that something will go wrong, and an experiment may have to be discarded because the electric current was off over night, or the air pumps were cut off for repair.

But these studies were not begun with a complete apparatus. The first experiments were conducted in 1927 with a pressure cooker, a bacteriological incubator, and an automobile pump. The results obtained were far from scientific, but they gave indubitable evidence that under the conditions of the experiments there was an acceleration of growth of the early chick embryo. To be sure this increased growth may have been due to higher humidity or it may have been due to elevated temperature, or finally it may have been due to the increased pressure which varied in a 24 hour period from 5 to 20 pounds.

A reserve air tank added later to the outfit aided in keeping the pressure up to 12-20 lbs. over night, but this did not materially change the results from those obtained when the pressure dropped to 5-8 lbs. during the night. The records of these early experiments were published in brief in the *Journal of the Elisha Mitchell Scientific Soc.* **42**: 188-192, 1927.

A large metal "incubator" built to contain a small commercial one was next added to the equipment. The smaller incubator did not prove successful and was replaced by a simple egg turner and the whole metal incubator was used as an incubating chamber. A description of this with its more recent additions has been printed in *Science* (**80**: 99-100, 1934). By the use of this improved apparatus it was not only possible to maintain a reasonably constant pressure, but much higher pressures could be used. This was limited by the pump to about 50 lbs., and would be limited by the incubator to about 75 lbs.

With the increase in pressure difficulties were experienced with electric thermo-regulators, heating elements, and fans. Ultimately heating elements and thermo-regulators were placed outside the incubator, and induction motor fans replaced the "brush" type within. Difficulties were also experienced with humidity controls. Finally one was devised using paper as an active agent and a mercury contact. This has proved very satisfactory.

The relative humidity had been calculated from the wet-dry bulb thermometers, but this has not been altogether satisfactory as the water evaporated from the "wet bulb" demands a considerable input and out-flow of air to keep the humidity at the desired level. More recently a new device has been constructed so that readings may be made from time to time although no provision has been made for automatically recording them.

Early check experiments in which this pressure incubator was run at normal pressures indicated that it was inferior at that time to commercial incubators, and the following results therefore are the more striking.

Some 94 eggs were incubated in this apparatus at 20 lbs., 29 at 22 lbs., and 35 at 40-50 lbs. While the average weight of the embryos taken on the 7th day was not markedly different from the average weight of the 79 controls, the minimum and more especially the maximum were pushed far beyond the controls. The normal incubator gave a typical curve with 75 embryos weighing between 0.480 g. and 0.720 g., with three below the minimum of this range and one above the maximum. The curve was exceedingly flattened at 20 lbs. pressure. Of the 94 embryos but 40 lay within the above limits and there were 24 below 0.480 g. and 30 above 0.720 g. Furthermore the maximum weight at 7 days for the control embryos was less than 0.760 g., while that for the pressure was more than 1.000 g.

At 45 lbs. pressure only 16 of the 35 embryos weighed between 0.480 g. and 0.720 g. while 4 weighed less and 15 weighed more, the minimum weight being less than 0.360 g. and the maximum more than 0.960.

Up to this time readings of temperatures were taken several times daily as no provision had been made for continuous recording either of temperature or humidity. Since there is some evidence that extremes of humidity do affect developmental rates in the chick it was highly desirable to control and record the humidity as well as the temperature within the pressure incubator. A wet-dry bulb recording thermometer was added to the apparatus.

The control of humidity as well as the control of temperatures by electrical contact under pressure offered difficult problems, as did the use of the "brush type" fan and metal heating elements. These were solved by using induction motors on the fans, by placing the heating elements and their controls outside the incubator, and by the use of a special paper humidity control with a mercury trip contact.

The problem of the recording of humidity has not been so easily solved. The wet-dry bulb recording thermometers (corrected for pressures) have not been wholly satisfactory since at times the evaporation from the wet bulb increases the humidity beyond the desired point. This can be balanced of course by a flow of dry air through the incubator, which in turn required more water to be put into the incubator. More recently a mechano-electric indicator has been added. This is non-recording, and therefore entails considerable additional attention to the apparatus.

With the improved apparatus additional runs have been made, and during the present season a very satisfactory experiment has been completed in which the conditions of temperature and humidity in the pressure incubator paralleled that of the controls closely and the pressure alone was the variable.

Eggs of the White Leghorn secured from Mr. L. G. Cheek, a local poultry man, were used in this experiment. Sixty-eight eggs were set in the pressure incubator at 35 lbs. and seventy-six were used for controls. The eggs were weighed and matched before incubation. Of the 68 pressure eggs 54 were alive when opened on the 9th day, and gave an average weight of 2.07 g. while 68 of the 76 controls were alive and gave an average weight of 1.458 g. An additional control of 63 eggs run in Mr. Cheek's incubators produced 51 live chicks with an average weight of 1.494 g. This gave the pressure chicks an increase in weight of about 42 per cent over the controls.

From cumulative data, but more especially from this last experiment, there can be little doubt but that increased atmospheric pressure does accelerate the development of the chick embryo up to the 9th day of incubation.

The question can well be raised whether the increase in the weight of the embryo might not be due to imbibition of water, since it was found that pressure eggs lost water more slowly than normal eggs at the same relative humidity. The 54 pressure eggs in the above experiments, after 9 days of incubation, had lost on an average of 2.1 g. while the 68 controls had lost 3.01 g. per egg, this in spite of the fact that the

relative humidity was the same for each group. Comparable observations had been made the preceding year.

It seemed that a critical test of the question could best be made by determining the dry matter of the pressure embryo and comparing that with the controls. This had been done previously in our laboratory by Mr. Blair, and while his results are consistent with the findings of this year they are not so striking, largely, it is believed, because he used eggs incubated at a lower pressure. The 54 pressure embryos, and 68 normal embryos mentioned above were individually desiccated at 95°C. to a constant weight, which for the former averaged 1.162 g. and for the latter 0.709 g. or an increase of 61 per cent in favor of the pressure chicks. The pressure chicks during desiccation lost 55.6 per cent of the original weight while the normals lost but 48.6 per cent. With such evidence it would be difficult to doubt that the increase in weight of the embryo in the pressure incubator was due to additional tissue produced under the stimulus of the compressed air.

The accelerating effect of pressure does not appear to be co-extensive with embryonic life. Nor is the early advantage maintained. Since the death rate of pressure chicks mounts rapidly about the tenth or eleventh day, an experiment was performed in which eggs were incubated under 30-35 pounds pressure for 9 days, after which they were transferred to a normal pressure incubator and incubated for a total of 14 days. When the 38 embryos were removed and compared with normal embryos there was no significant difference in their weights. Similarly, eggs which have been incubated for 18-19 days under pressure when removed to a normal pressure incubator and allowed to hatch show no significant difference in the weight immediately after hatching; 23 newly hatched pressure chicks averaging 44.95 g. while 30 controls averaged 44.43 g.

There does seem to be on the other hand some effect on post-hatching growth. In one series of experiments 23 pressure chicks weighed 44.95 g. at hatching. Nineteen of these which survived to 21 days post-hatching averaged 113.8 g. while 47 controls averaged 44.6 g. at hatching and 45 survivors averaged 97.0 g. at 21 days.

This advantage in favor of the pressure chicks might be thought to be due to the survival under pressure of only the strongest and most virile chicks. Selecting the 9 largest males of the controls which averaged 117.3 g., and comparing them with the 9 surviving pressure males which averaged 130.5 g., it is evident that even under the best of condi-

tions for the controls the gain is 12.5 per cent; sufficient, it is believed, to be significant.

The mode of action is not so clear, but there are at least two possibilities which seem reasonable. It has been pointed out that increased humidity accelerates development. Now while the relative humidity in the pressure incubator has been held at the same level as in the control incubator, the water has not left the egg as rapidly and there is simulated a condition of high humidity. The increase in weight here however is much greater than can be obtained by optimum humidities. The critical test, i.e. regulating the humidity in the pressure incubator so that the water loss is equivalent to that under normal incubation, has not been made.

The second plausible explanation of the increased weight is that the metabolic level is raised by the increased oxygen pressure. This is not supported by any experimental evidence from this study. While there is no direct evidence to support this view it seems a little more reasonable to accept both as causal factors in the acceleration of growth in embryonic chicks under increased atmospheric pressure.

HETEROTHALLISM AND STERILITY IN *ACHLYA* AND OBSERVATIONS ON THE CYTOLOGY OF *ACHLYA* BISEXUALIS*

By JOHN ROBERT RAPER

PLATES 22-24

INTRODUCTION

Weston (1917) described a sexually sterile form of *Achlya* which produced heavy-walled resistant spores. The vegetative and sporangial development of the fungus agreed with other members of the genus, as *A. americana*, but in its further development the fungus differed from other *Achlyas* in that zoosporangium formation was followed normally by the production not of sexual organs, but of spherical heavy-walled resistant spores. The resistant spores averaged $110\ \mu$ in diameter, the size being apparently correlated with the vigor of the mycelium. In water the resistant spores germinated to form zoosporangia while in nutrient solutions mycelia were formed directly. Weston tried to induce the plant to form sexual organs by growing it in different chemical and nutrient solutions but without success.

Coker (1923) found and described what he considered to be the same plant and called it simply *Achlya* sp., form without oogonia. The resistant spores of Weston he considered as gemmae, but does not mention the fact that they are more heavy-walled than the gemmae. He was also unable to induce the formation of sexual bodies by varying the food supply and the external conditions.

Coker (1927) reported, from the unpublished notes of A. B. Couch, that heterothallism had been shown to exist in a form of *Achlya*, which he says, to all appearance, is the same plant as described in "The Saprolegniaceae" (1923) as *Achlya* sp., form without oogonia. He named the plant *A. bisexualis*.

The present study was undertaken to determine the extent of heterothallism in the genus *Achlya* and to investigate the cytology of the

* A thesis submitted to the Faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Master of Arts in the Department of Botany.

resistant spores and the sexual organs of *A. bisexualis*. This study was suggested and made under the supervision of Dr. J. N. Couch and Dr. W. C. Coker to whom the writer wishes to express a sincere appreciation for all the help and suggestions given by them. The writer also wishes to thank Miss Ruby Rice, Miss Mary Vardell, E. V. Deans, and Leland Shanor for collections and cultures of sterile *Achlya*.

MATERIALS AND METHODS

Samples of 25–50 cc. of water from streams, springs, or ponds, along with some of the sediment, were collected and brought into the laboratory. Soil collections of 5–10 cc. were also used as a source of material.

The isolations were made in the following manner: The collections were poured into sterilized Petri dishes, and distilled water (which had previously been treated with animal charcoal, filtered, and sterilized in the autoclave) was added. Into each of these dishes were dropped two or three halves of boiled hemp seed. The fungal growths on the hemp seed were examined at intervals of about 24 hours after their appearance and a single hyphal tip of any *Achlya* which appeared to be sterile was transferred to a plate of maltose-peptone agar (maltose, 0.3%; peptone, 0.1%, agar, 2.0%). After 2–4 days' growth the periphery of the resultant mycelium was usually quite free from bacteria. From the outer edge of the radial growth a few hyphal tips were cut off in a small square of agar and this was transferred to a drop of sterile water. The hyphae in the agar grew out into the water for a short distance and produced sporangia at their tips. These, on maturing, liberated a large number of zoospores in 6–8 hours after being placed in the water. A small drop of this water, containing a number of spores, was then streaked on an agar plate. After allowing a few hours for the spores to germinate, the plate was examined under a dissecting binocular microscope and the position of isolated spores marked. A day after streaking the spores on agar, the mycelia resulting from their germination were large enough to enable one to cut out small squares of agar containing a number of hyphal tips from their periphery using the microscope and a sharpened spear-head needle. These squares were then transferred to sterilized Petri dishes where half a boiled hemp seed was placed on each and enough sterile water added to cover the bottom of the dishes, yet of insufficient quantity to float the seed from the agar. The growth on the hemp seed became visible to the naked eye after 4–8 hours. Cultures were washed frequently during the actively growing period, i.e., for the first four or five days after transfer.

Stock cultures of all the strains have been cultured in sterile water on hemp seed, and have been washed at intervals of 2-3 weeks, fresh hemp seed being added whenever necessary. They have also been carried through the procedure for isolation described above at intervals of about 6 weeks to insure their purity.

Cultures for crosses have been made by adding hemp seed to agar squares taken from a clean mycelium of the desired strain on nutrient agar. The young plants were washed daily until old enough to be used in the crosses, i.e., until zoosporangial production was well under way and the vegetative growth was approaching maturity. This stage of development was usually reached 2-4 days after transfer to the hemp seed. The pair to be tested was then placed in a sterilized Petri dish with their hyphal tips barely touching and enough sterile water was added to keep them wet but not enough to float them apart or too close together. In the latter case, subsequent observations would have been rendered much more difficult due to the density of growth in the region of contact of the two mycelia. After sufficient intermingling had taken place to cause the mycelia to hold together, enough additional water was added to make about 3-4 mm. in the bottom of the dish.

Early in this work it was only by repeated transfers through several changes of agar that material was obtained which was entirely free from bacteria. Later a method has been used by which the fungus can be completely isolated from its bacterial contamination with far less effort and in a fraction of the time formerly required. Very small glass beads ($1/3$ - $1/2$ mm. in diameter) are fused to the edge of a small glass circle, such as is used in making a van Tieghem cell. The circle is then placed in the bottom of a sterilized Petri dish, the glass beads preventing the circle from resting on the bottom of the dish. The nutrient agar is then poured into the Petri dish to a depth which brings its surface well up on the sides of the glass circle.

After the agar has solidified the plate is inoculated by transferring a few hyphal tips, sporangia, or resistant spores to the area of agar lying inside the glass circle. As growth takes place, the hyphae grow down through the agar and some of them grow under the glass circle. In growing down through the agar, the bacteria are left at the surface of the agar inside of the circle and that portion of the mycelium which lies outside of the circle is free from bacteria. To test the absence of bacteria in this portion of the mycelium, small squares of agar, containing a number of hyphal tips, were cut off from the edge of the mycelium and each was placed in a small flask of maltose-peptone solution (strength

same as in the agar used for isolation). A control of a small square from a culture known to be contaminated was treated in an identical manner. In the flasks containing the squares to be tested no clouding of the liquid occurred, a delicate spherical mycelium forming from the inoculum in the agar. This method has been tried on a number of cultures of other species badly polluted with bacteria and in all cases so far has proved successful.*

STRAINS OF STERILE *ACHLYA*

During a period of 18 months approximately 500 collections have been made. From these collections 32 cultures of sterile *Achlya* have been isolated. Most of the collections were made from the vicinity of Chapel Hill, N. C.; additional collections, however, have been made at Murphy, N. C., Welcome, N. C., Maryville, Tenn., and Baltimore, Md. All of the sterile forms with the exception of three (Nos. 26 from Baltimore, Md., and 19 and 27 from Maryville, Tenn.) were isolated from collections from Chapel Hill and vicinity.

Of the 32 strains, 8 have been shown to belong to the female strain of *Achlya bisexualis*, 7, males of the same species, 12 are occasional hermaphroditic forms of *A. bisexualis*, but in the presence of a male plant give a typical female reaction. The remaining 5 cultures have given no reaction whatever when crossed with any of the other plants. Abbreviated descriptions of the strains follow:

Achlya bisexualis:

A. Female strain:

Mycelium rather large; hyphae moderately large and tapering; sporangia normal for the genus; cylindrical to oblong gemmae produced in abundance; numerous terminal resistant spores produced either singly or in chains (figs. 1, 2, 27, and 34).

B. Hermaphroditic-female strain:

Vegetative growth indistinguishable from that of female plant. Abortive oogonia are produced occasionally in small restricted areas of the mycelium, accompanied by undeveloped antheridia (fig. 29).

C. Male strain:

Growth more extensive but thinner than in female strain; hyphae more delicate; gemmae typically oblong. Small hyphae common; re-

* Since this paper was received for publication, a paper by Blank and Tiffney has appeared describing a method of preventing the growth of bacteria in cultures of *Saprolegnia* by ultra-violet rays (*Mycologia* 28: 324. 1936).—Ed.

sistant spores always lacking. A large portion of the sporangia in older plants being of a pseudo-dictiosporangial type (figs. 3, 4, 5, 28, and 33).

Morphological differences between male and female strains: (1) Smaller size of the female; (2) longer but thinner hyphae of the male; (3) presence of pseudo-dictiosporangia in male; and (4) the presence of the resistant spores on the female and their absence on the male.

The five collections which have remained sterile are quite different from either of the three strains of *A. bisexualis* and apparently belong to four different species. An abbreviated description will be given of each strain.

Sterile Form 1. Small, slow-growing; densely matted. Hyphae stout at base, tapering sharply to tip. Sporangia in cultures 4-5 days old and older, often pseudo-dictiosporangial type. Gemmae produced by the segmentation of the vegetative hyphae, hence cylindrical (figs. 6 and 31). Two collections: 28 and 31.

Sterile Form 2. Growth thin and extensive; growth reaching approximately twice the size of the preceding form. Hyphae large. Sporangia normal for the genus. Gemmae formed by the septation of the vegetative hyphae and sometimes up to 1.15 mm. in length (fig. 30). A single collection: 30.

Sterile Form 3. Small thin mycelium; individual hyphae moderately large. Among the spores are frequently found large masses, measuring up to 40 μ , which result from the incomplete segmentation of the contents of the sporangium. A single collection: 32.

Sterile Form 4. Mycelium very thin and extensive, often reaching a diameter of 6 cm. in water culture on hemp seed; sporangia in young cultures are typical in form and dehiscence for the genus *Achlya*, but in older cultures practically all of the spores are liberated by the disintegration of the sporangial wall in a typical *Thraustotheca* manner (figs. 7, 32, and 35). A single collection: 29.

These strains are of some interest as they are the first collections of *Achlya* lacking sexual organs to be described which do not agree in all particulars with the original plant of Weston, which was doubtless a female plant of *A. bisexualis*. It is possible that these forms will eventually be shown to be sexual strains of other species of *Achlya*.

STUDIES IN HETEROTHALLISM

With the exception of the five most recently collected plants, 19, 27, 30, 31, and 32, all possible crosses have been made between the cultures. In the case of the five, each has been crossed with at least two males, two females, and each of the sterile plants.

The results of these crosses are shown in the two accompanying tables, the first being the results of pairings of true females with males, the second, the results of crosses between males and hermaphroditic-females, which in their reaction with males are identical with the true females.

TABLE I

Summary table of crosses between true females and males of A. bisexualis

FEMALE STRAINS	MALE STRAINS						
	21	22	23	24	25	26	2
1	II	—	—	I	—	—	0
5	III	I	I	I	—	—	0
9	I	—	—	—	II	—	0
10	—	—	—	II	—	—	II
15	II	—	—	II	—	—	0
16	II	—	—	II	—	—	0
18	I	—	—	II	II	—	II
19	0	0	0	0	II	0	III

TABLE II

Summary table of crosses between hermaphroditic-females and males of A. bisexualis

HERMAPHRODITIC-FEMALE STRAINS	MALE STRAINS						
	21	22	23	24	25	26	27
2	II	—	—	—	I	I	III
3	III	—	—	I	—	—	0
4	II	—	—	I	—	—	0
6	I	—	—	—	—	—	0
7	II	—	—	—	—	—	0
8	I	—	—	II	—	—	0
11	III	—	—	III	—	—	0
12	II	—	—	II	II	II	III
13	I	—	—	I	—	—	0
14	III	—	I	—	—	—	0
17	II	II	—	II	—	—	0
20	III	—	—	—	—	—	0

In the tables, the Roman numerals indicate the relative abundance of sexual organs found in the matings: "III" indicates the presence of numerous oogonia along the line of intermingling of the hyphae from the two mycelia (in no case have the oogonia been plentiful enough to show as a distinct line to the naked eye); "I" indicates the presence of very few sexual organs; and "II" is an intermediate grade. Minus signs

("—") mark those crosses in which no reaction occurred between the male and the female plants. In a number of cases (mainly with the two sexual strains most recently collected (19 and 27), no crosses have been made; zeros ("0") indicate such cases.

From a comparison of the results recorded in the two tables it is evident that the reaction between males and females was, at best, rather uncertain. Two males, 21 and 24, constantly gave better reactions than any of the remaining males with the possible exception of 27, for which only five of the possible twenty matings with female or hermaphroditic-female plants have been made. In those five crosses in which 27 has been used the reactions have been more nearly constant than has been the case when any other male was crossed with a number of females; also, in these same crosses the percentage of eggs which have matured has been definitely above the average for all crosses.

A marked variation in the sexual strength of certain females has also been evident. No female, however, seemed to be very constant in its reaction, but of the twenty, 5 and 12 have perhaps been the best; 19, from Maryville, Tenn., only used in a very few crosses, have in those crosses given relatively constant reactions.

The sexual reaction between male and female plants of *A. bisexualis* may be described as follows: in the region where the hyphae of the two mycelia intermingle, oogonia appear on the hyphae of the female plant. During their formation very small branches, the antheridial hyphae, arise from the main hyphae of the male plant and become applied to the sides of the oogonia (fig. 8). Also many of the antheridial hyphae twine around the vegetative hyphae of the female plant as well as the oogonial stalks, frequently forming complex gnarls of hyphae (fig. 9). A large number of antheridial branches are commonly found on each oogonium. The antheridia may be simple or branched and are frequently finger-like in shape, being applied to the oogonia by their sides or ends.

Most of the oogonia are borne on lateral branches of the main hyphae, but some terminal ones are commonly produced. Terminal resistant spores have been seen which functioned as oogonia, antheridia being applied to their sides and the contents dividing to form a number of eggs (fig. 10). This, however, is rare.

It may be said, however, that no matings could be called satisfactory. In all of the crosses the number of oogonia was somewhat less than hoped for, and the percentage of eggs which disintegrated before reaching maturity was exceedingly high. Even in the crosses which pro-

duced the greatest number of oogonia they were not plentiful enough to be visible to the naked eye as a line, a condition often seen in crosses of another heterothallic Oomycete, *Dictyuchus monosporus*, described by Couch (1926). Also a far smaller percentage of the eggs formed reach maturity in *A. bisexualis* than in that species, in which, however, not nearly all of the eggs mature in crosses made under cultural conditions.

A consideration of this inefficiency in producing large numbers of oogonia and germinable eggs brings certain important questions to mind. The foremost of these is whether or not the plant shows such lack of proficiency in nature. It is probable that it does not and if this sexual weakness only occurs in culture there are two plausible explanations: (1) the failure to reproduce in the laboratory the cultural conditions necessary for normal sexual development, and (2) the failure to collect perfectly compatible strains.

It is even quite possible that the male and female strains described, and with which this work has been done, do not belong to the same species, considering the fact that so very few eggs mature and that the sexual reaction in general is so weak. It may possibly be only a case of incomplete hybridization as in matings of strains of different species of the Mucorales, as was first demonstrated by Blakeslee (1904).

A further consideration is the fact that all of the male and female plants with the exception of three are from collections made from a very small area, approximately 20 square miles. In all probability a number of collections from a wider range would yield strains that would be more compatible with each other and in whose reactions the efficiency of sexual reproduction would be greatly increased.

CULTURAL VARIATIONS

Both male and female strains of *A. bisexualis* are exceedingly variable in culture; the female or hermaphroditic-female, however, shows greater variations than the male. The resistant spores, so common and characteristic of the female and hermaphroditic-female strains, are not produced in hot weather (their production never occurring above a temperature of 30°C). Also the occasional occurrence of sexual organs on the hermaphroditic-female plants suggested the possibilities of cultural conditions affecting their production.

In an effort to induce the hermaphroditic-female plant to produce oogonia regularly in culture and to study the effects of nutrients and salts on the production of resistant spores, no. 8 ♀, a typical plant of

that strain, was grown in 45 different solutions of haemoglobin, leucin, peptone, levulose, glucose, saccharose, potassium phosphate, mono-potassium acid phosphate, di-sodium acid phosphate, sodium bisulphite, ammonium tartrate, calcium phosphate, ammonium nitrate, sodium chloride, and potassium nitrate. The variations in growth and production of gemmae and resistant spores were as great as expected. No correlation between the relative growth of the plant and the abundance of gemmae and resistant spores could be established, nor did the production of gemmae and resistant spores, as a rule, parallel each other.

In certain solutions whose dissolved content was as high as 0.25% or higher, numerous dictiosporangia were formed. Klebs (1899) showed that variation in sporangial dehiscence could be brought about by changes in the concentration of the medium. In one solution, 0.375% glucose, an interesting intercalary variation of the resistant spore was found, which consisted of a group of three bodies, the two end ones spherical and the larger central one barrel-shaped. In only two of the solutions were any oogonia produced and in these there were very few, formed only in small restricted areas as typical for the hermaphroditic-female plant when grown under more nearly normal conditions.

CYTOLOGICAL OBSERVATIONS

Material to be used for cytological preparations was killed and fixed in (1) Claussen's fixative or (2) chromic acid—formalin solution (chromic acid, 1.25 grams, formalin (commercial 40%) 10 cc., and water, 200 cc.) for 6–10 hours. It was then washed for 12 hours, dehydrated, cleared, imbedded in paraffin in the usual manner, and sectioned 7.5–10 μ in thickness. The stains used were: (1) Gram's iodine method as described by Couch (1932); (2) the same stain with a slight counterstain of eosin; and (3) Heidenhain's iron-alum-haematoxylin method. Gram's stain was the most satisfactory of the three.

1. Vegetative hyphae.

The wall of the vegetative hypha is thin, 0.3–0.7 μ . A thin layer of granular vacuolated cytoplasm everywhere lines the wall to a thickness of 2–7 μ and in which numerous nuclei are scattered at irregular intervals (fig. 11, a & b). The vegetative nucleus consists of a conspicuous nucleolus, an area of clear nuclear sap, and a delimiting nuclear membrane. Along the nuclear membrane lie a variable number of dark staining granules from which extend very delicate threads to the nucleolus. The nuclei range in size from 3.1–3.5 μ , the average being 3.3 μ .

2. Resistant spores.

The resistant spores are characterized by their spherical shape and thick hyaline wall which varies in thickness from 1.5 to 2.2 μ according to the age of the body. The contents consist of dense, granular, vacuolated cytoplasm and numerous scattered nuclei (as many as 250 having been counted in a single "spore"). The wall of the mature resistant spore is made up of two distinct layers: an inner layer surrounding the individual spore and an outer layer which surrounds the entire chain of spores, lying in all places in close contact with the inner layer. In addition to these there is what appears to be a layer of gelatinous secretion which accumulates on the exterior of the resistant spore as it ages (figs. 12, 13).

The nuclei of the resistant spore are identical with those of the vegetative hypha in structure and size.

A marked change occurs in the cytoplasm of the resistant spore after it has been cut off from the parent hypha. In the young stages the cytoplasm appears to be identical to that of the hypha, being granular and containing numerous small vacuoles. In a slightly older stage numerous, very small, spherical, darkly staining bodies have appeared. As the "spore" ages these granules enlarge, and eventually attain the size of the nuclei (fig. 14, a-d). These granules are probably food inclusions of some sort and give the same appearance as similarly staining granules which lie in the cytoplasm of the zoospore. At the same time another change is taking place. The cytoplasm which, at an early stage, was finely granular has become more coarsely granular and the definite limitations of the scattered vacuoles have been lost.

In the "spores" of all ages there are numerous small rod-shaped, straight or bent, and spherical granules which stain dark with Heidenhain's haematoxylin (fig. 14e). These are probably chondriosomes; however, no special technique to show these bodies has been used as a check.

What is the true nature and function of the resistant spores? This problem has been evident since these bodies were first discovered by Weston (1917), who considered them to be wholly vegetative in function as a compensation for the supposed lack of a sexual phase in the life history of the fungus. The fact that they occasionally behave as female sexual organs has been stated above, and it is conceivable that this is their primary function and that due to the heterothallic nature of the plant and the possibility of frequent isolation of the female from the male element they have developed the ability to remain as vegetative resting bodies when the male element is not at hand. In the event

of the two sexual strains coming in contact, in some cases, the contents probably undergo the various phenomena that takes place in the oogonium when its contents are differentiated into a number of uninucleate eggs. As the functioning of the resistant spore is so rare, we were not fortunate enough to get any cytological evidence as to what goes on in such cases. Although these bodies are originally formed in the same manner as similar structures of *Saprolegnia dioica* described by Walz (1870), they have been shown to exhibit greater versatility of function by occasionally acting as female sexual organs.

Cultures of a typical female having abundant "resistant spores" were tested to determine the resistance, if any, of the spores to desiccation. Small masses of hyphae and terminal spores were put on filter paper and allowed to dry in air. No spores could be germinated after the filter paper on which they were placed became dry. The spores are thus perhaps no more resistant to desiccation than vegetative hyphae or gemmae.

3. Gemmae.

The gemmae are obviously only accumulations of cytoplasm in portions of the vegetative hypha, as the wall of the gemma is no thicker than that of the hypha. The lining layer of cytoplasm, however, is much thicker and contains a proportionately greater number of nuclei than the hypha. The cytoplasm and nuclei appear to be identical with that of the vegetative hypha (fig. 15).

4. Sexual organs.

Due to the small number of sexual organs available for study, the cytological story here is incomplete in many details and the present account is considered as preliminary.

The oogonium, a spherical body, contains a peripheral layer of finely granular, vacuolated cytoplasm and 50-100 nuclei which lie in the cytoplasm in a more or less regular manner, the distance between nuclei usually being practically the same. Antheridia numbering from one to several lie in close contact with the oogonial wall (fig. 16).

The wall of the oogonium is somewhat thicker than that of the vegetative hypha, but not so thick as that of the resistant spore. It is smooth, unpitted, and averages $0.7-1.5 \mu$ in thickness. The wall of the antheridium is exceedingly thin, certainly being no thicker than a hyphal wall.

The nuclei of the young oogonium are identical with those of the vegetative hypha, each consisting of chromatin granules, a nucleolus, a region of nuclear sap, and a delimiting membrane. The nuclei of the

antheridia are at this stage, and during the following changes, in a similar condition to those inside the oogonium. In an older stage a definite enlargement of the nuclei of both sexual organs occurs. The granules on the nuclear membrane become more conspicuous and a contorted structure, which is evidently the spireme of the early stage in mitosis, lies in the nuclear sap. The nucleolus is also considerably enlarged, and in some cases, apparently divided into two smaller darkly staining bodies (fig. 17). No change is apparent in the cytoplasm.

From the preparations which have been studied, it appears that the nuclear membrane now disappears. The spireme breaks transversely to form a number of distinct chromosomes (fig. 18). Most of the nuclei now begin to disintegrate, the chromosomes and nucleolus becoming much smaller and less distinct, a similar reaction taking place in the antheridium. A small number of nuclei in each oogonium, however, complete their mitotic division (fig. 19). Several mitotic figures have been seen in each of a number of oogonia.

A detailed study of a mitotic figure shows a typical spindle having a distinct dark centrosome at either pole and a number of chromosomes lying along the fibers between (fig. 20, a and b). The chromosomes are irregular in shape. The number of chromosomes could not be determined with certainty due to their exceedingly small size, but as many as eight have definitely been seen.

The formation of eggs in *A. bisexualis* is in all probability like that described by Trow (1899 and 1904), Mücke (1908), Claussen (1908), Davis (1903), and others for various species of the *Saprolegniaceae*, but unfortunately no stages have been found. However, eggs which had evidently recently been formed have been seen in large numbers. In the young egg the membrane is thin and numerous small vacuoles are scattered in the cytoplasm. The vacuoles here apparently represent the oil droplets in the living condition, the oil having been dissolved out in preparation by the clearing agents (figs. 21, 22). As the eggs mature these vacuoles coalesce to form larger vacuoles.

Numerous oogonia have been found which had one or more fertilization tubes extending into them from the accompanying antheridia (figs. 22 and 23). Every fertilization tube that has been observed has always contained a nucleus in its tip. The oogonial wall at the point of entrance of the fertilization tube is slightly raised.

It appears that fertilization takes place, although no cases were seen where the antheridial tube was clearly connected with the egg. A number of eggs, however, were seen which contained two nuclei (fig. 24),

one lying in the center of the egg, supposedly the female nucleus, and another at some distance from it. Other stages were seen which appeared to be fusion stages (fig. 25), but this could not be determined with assurance.

The mature, or nearly mature, egg is surrounded by a thick wall which is composed of two layers. The wall of the egg is strikingly similar to that of the resistant spore. There is, however, no sign of the outer "secretion" layer as in that body. There is a single large vacuole in the mature egg, it being, supposedly, the end result of the fusion of the many smaller vacuoles of the younger egg and represents the position of the eccentric oil drop seen in the egg in the living condition. The cytoplasm of the nearly mature egg lies around the vacuole, but it is accumulated into denser masses on the side opposite the eccentrically located vacuole. A single large nucleus lies near the center of the cell at the edge of the vacuole (fig. 26).

SUMMARY

1. A new method for the isolation of water fungi from bacterial contamination is described.

2. *A. bisexualis* is a relatively uncommon plant, only 27 plants having been found in 500 collections.

3. Plants of *Achlya bisexualis* are shown to belong to three morphologically distinct strains: male, female, and hermaphroditic-female. An abbreviated description is given of each strain and certain morphological differences between male and female strains are pointed out. Aside from the occasional production of abortive oogonia and antheridia by the hermaphroditic-female plants, this strain is indistinguishable from the female.

4. Four additional strains of sterile *Achlya* have been collected and are here briefly described. They are different from any of the three strains of *A. bisexualis* and are perhaps sexual strains of other heterothallic species of *Achlya*.

5. Vegetative variations of the hermaphroditic-female strain of *A. bisexualis* have been induced by the use of various solutions of nutrients and salts, but the regular production of sexual organs has not been accomplished.

6. A cytological study of the resistant spores of the female and hermaphroditic-female strains of *A. bisexualis* is given. The resistant spore, so far as structure is concerned, more nearly resembles the vegetative structures of the fungus than the oogonium. The latter was

thought probable because of the fact that the resistant spore occasionally functions as an oogonium.

7. Cytological observations on the sexual organs of *A. bisexualis* agree with accounts of work on other members of the *Saprolegniaceae*.

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PLATE 22

Achlya bisexualis

Figs. 1-5

Fig. 1. Resistant spores of female plant. $\times 42$.

Fig. 2. Germinating resistant spore showing sporangium at tip of germination tube. $\times 75$.

Fig. 3. Tip of pseudo-dictiosporangium of male plant. $\times 395$.

Fig. 4. Stages in spore emergence from pseudo-dictiosporangium; cysts remaining in sporangium form a false net. $\times 795$.

Fig. 5. Typical gemmae of male plant. $\times 65$.

Achlya, sterile forms

Figs. 6-7

- Fig. 6. Typical gemmae and normal *Achlya* sporangium of Sterile Form 1. $\times 42$.
Fig. 7. Hyphal tip of Sterile Form 4, showing a normal *Achlya* sporangium and one whose wall is disintegrating in a typical *Thraustotheca* manner. $\times 115$.

Achlya bisexualis

Figs. 8-29

- Fig. 8. Young oogonium and antheridial branches. $\times 200$.
Fig. 9. Young oogonium and oogonial stalk surrounded by antheridial hyphae, a phenomenon frequently seen in the species. $\times 42$.
Fig. 10. A former resistant spore, the contents of which have become differentiated into a number of eggs, apparently serving the function of an oogonium. Optical section. $\times 42$.
Fig. 11. Transverse section of a vegetative hypha. $\times 1197$. Also vegetative nuclei showing detailed structure. $\times 2015$.
Fig. 12. A chain of resistant spores in longitudinal section. $\times 180$.
Fig. 13. A detailed study of the wall structure at the point where the two upper resistant spores of Fig. 12 are in contact. $\times 795$.
Fig. 14. Portions of resistant spores shown in Fig. 12; *a* is the spore at lower end of chain; *b*, the second from the end, etc. Note progressive thickening of wall and "growth" of the dark-staining granules; *c* is a portion of another resistant spore of the same stage as *d*, but stained with Heidenhain's haematoxylin. $\times 795$.

PLATE 23

- Fig. 15. Longitudinal section of a gemma and adjacent portions of hypha. $\times 395$.
Fig. 16. Young oogonium and four antheridial branches; nuclei of both sexual organs in resting stage. $\times 795$.
Fig. 17. Slightly older oogonium than shown in Fig. 16; nuclei in early prophase of mitosis. $\times 795$.
Fig. 18. Oogonium; nuclei in late prophase of division, the spireme having divided into a number of distinct chromosomes. $\times 795$.
Fig. 19. Oogonium; six nuclei beginning to disintegrate; two others in late metaphase of mitosis. $\times 795$.
Fig. 20. Detail of mitotic figures; *a* is the larger of the two mitotic figures of oogonium shown in Fig. 19; *b* is from another oogonium. $\times 2015$.
Fig. 21. Young egg; membrane thin, numerous small vacuoles. $\times 795$.
Fig. 22. Oogonial wall pierced by fertilization tubes. $\times 795$.
Fig. 23. An oogonium into which a fertilization tube extends from one of the adherent antheridia; nucleus in extreme end of tube. Nuclei of two eggs in adjoining section. $\times 795$.
Fig. 24. Fertilized egg; female nucleus in center and male nucleus a short distance away. $\times 795$.
Fig. 25. A stage in the fusion of the male and female nuclei. $\times 795$.
Fig. 26. Nearly mature egg surrounded by thick two-layered wall. The numerous vacuoles of earlier stages have coalesced to form a single large eccentric vacuole. $\times 795$.

PLATE 24

Achlya bisexualis

Figs. 27-29

Fig. 27. Female plant. $\times 1$.

Fig. 28. Male plant. $\times 1$.

Fig. 29. Hermaphroditic-female plant. $\times 1$.

Achlya, Sterile forms

Figs. 30-32

Fig. 30. *Achlya*, Sterile Form 2. $\times 1$.

Fig. 31. *Achlya*, Sterile Form 1. $\times 1$.

Fig. 32. *Achlya*, Sterile Form 4. $\times 1$.

Achlya bisexualis

Figs. 33-34

Fig. 33. Pseudo-dictiosporangium of male plant. $\times 100$.

Fig. 34. Resistant spores of female. $\times 100$.

Achlya, Sterile form 4

Fig. 35. Sporangia of *Achlya*, dehiscing in a typical *Thraustotheca* manner. $\times 100$.

PLATE 22

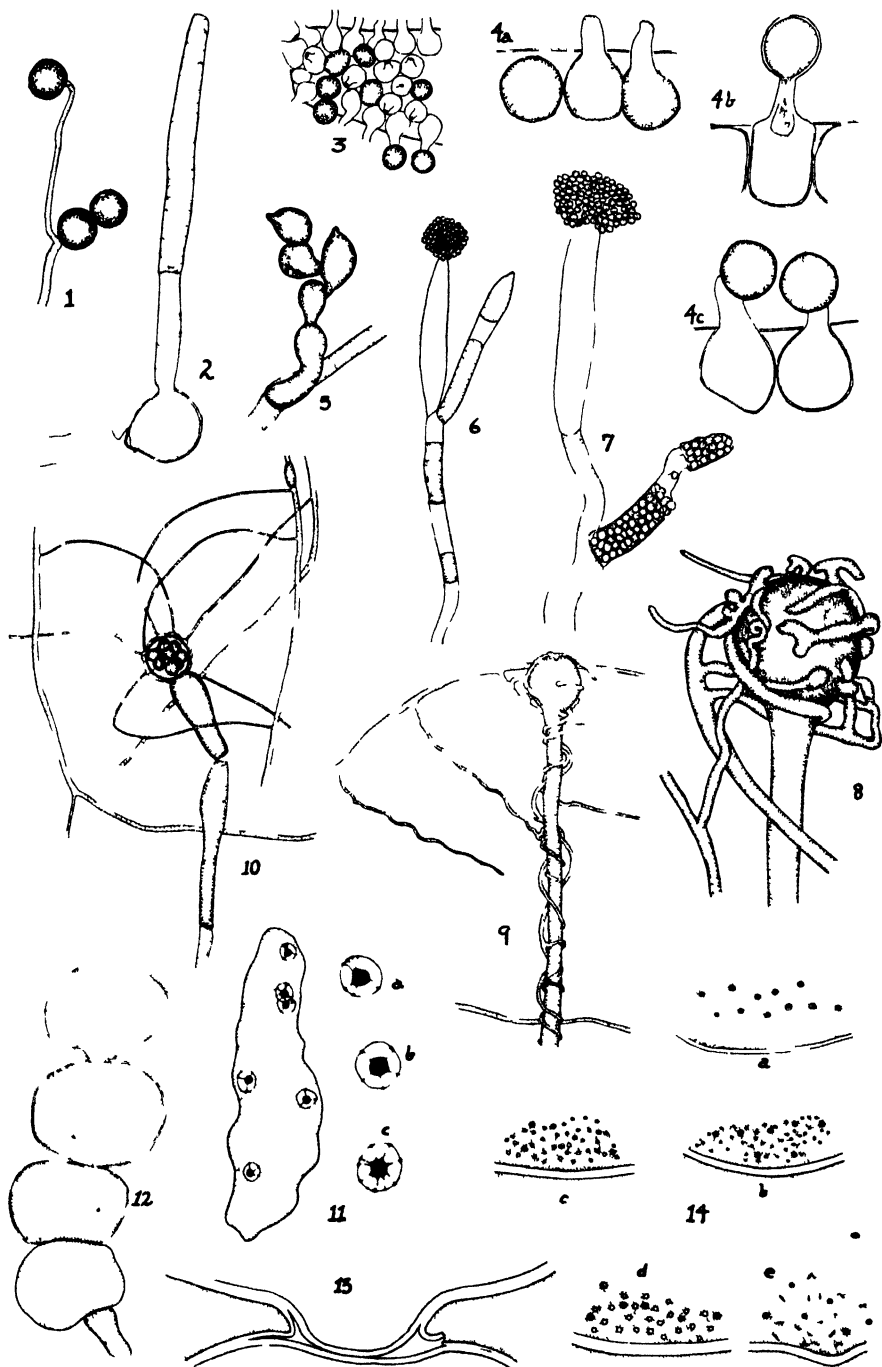


PLATE 23

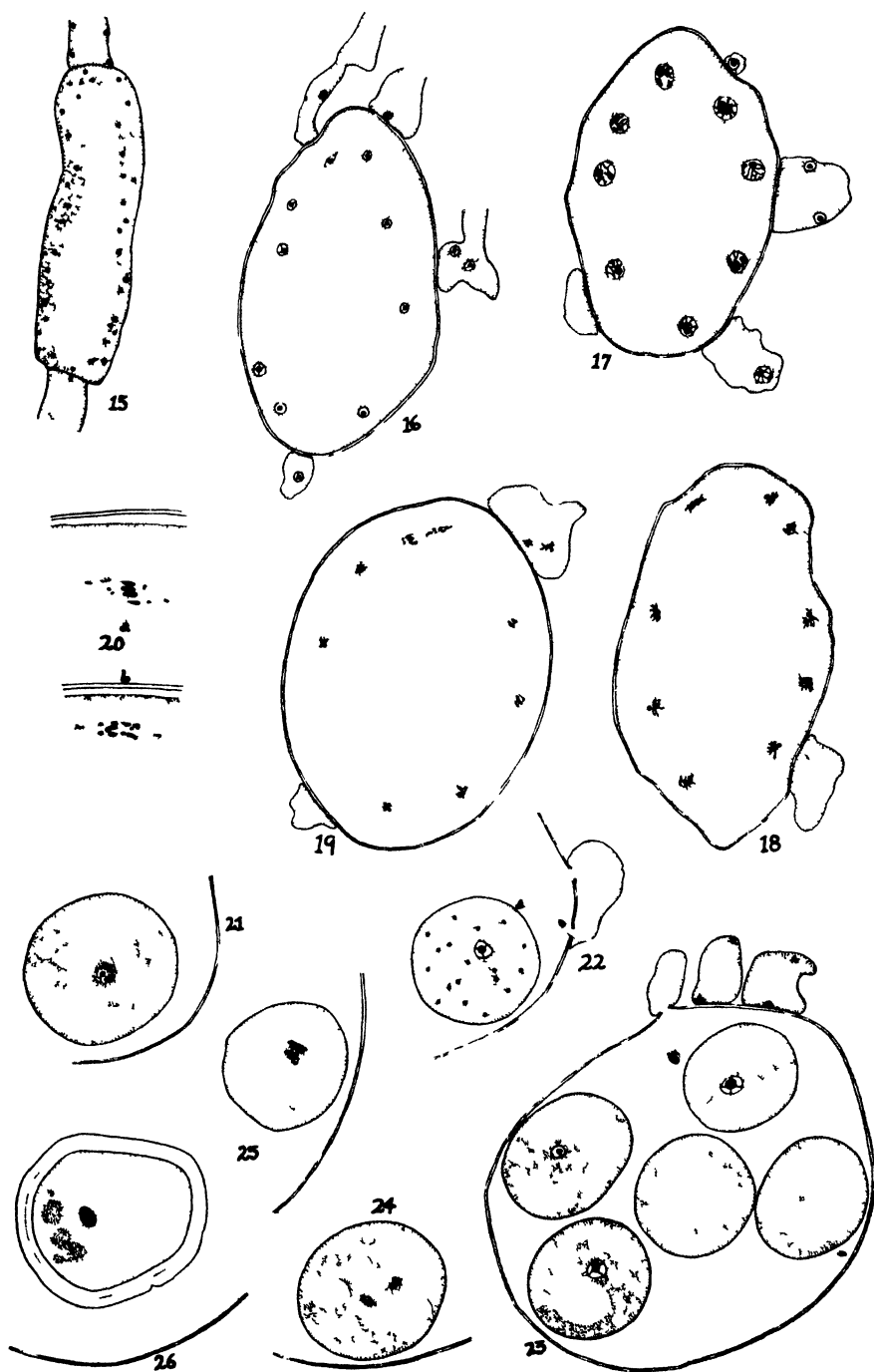


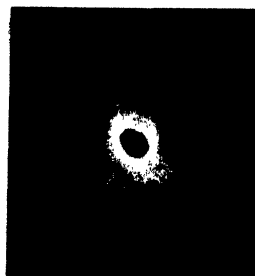
PLATE 24



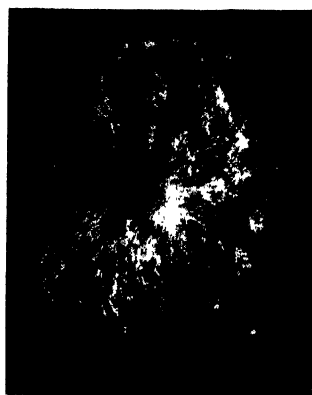
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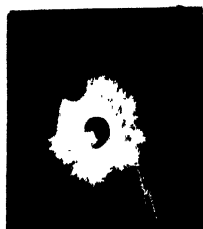
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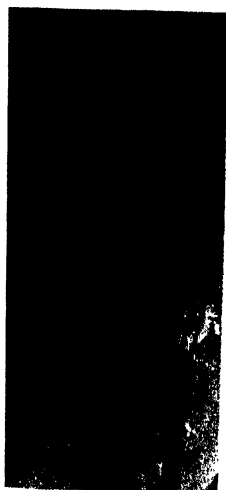
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35



34

A NEW SPECIES OF RHIPIDIUM FROM MOUNTAIN LAKE, VIRGINIA

By VELMA D. MATTHEWS

PLATE 25

While examining a number of ripe huckleberry and *Amelanchier* fruits which had been lying for some time in the water of Mountain Lake, Giles County, Virginia, the writer noted a few fruits with very small clumps of fungus growth which proved to be a *Rhipidium* that is apparently undescribed. All the specimens of the new species were small, the longest main trunk (at least a thousand individual plants must have been examined) measured only 214 μ long, which is much shorter than the measurements given for *R. parthenosporum* Kanouse, which it resembles in the presence of the very short secondary branches which bear the sporangia and oogonia.

In the laboratory the sporangia formed zoospores only on a few occasions and then only after many attempts on the part of the writer to induce their formation. On one day several plants bearing many young sporangia were dissected out from the substratum about 9:30 A.M. and placed in a hanging drop of boiled lake water, which was changed several times during the day. By 8:00 P.M. many of the sporangia were forming zoospores, which escaped by the whole group pushing out in a mass, the individual spores then soon separating and swimming away as very active reniform, biciliate zoospores, containing a large vacuole and many large glistening granules. These zoospores in the hanging drop germinated within twelve hours by the formation of a tube, which branched very near its origin. Many attempts were made to grow this species in pure culture, but within the course of the three weeks available no growth of *Rhipidium* was found on the new cultures.

Oogonia mixed with the sporangia were rarely found on material just brought in, but developed in large numbers on plants kept in the laboratory for several weeks. These oogonia were similar to those in the other species of *Rhipidium* except that in many cases the thin wall had minute papillae. At the point where the antheridium comes in contact

with the oogonium a bulging of the oogonial wall was noted in several cases, which suggests a receptive papilla.

Rhipidium compactum n. sp. may be separated from all other species of *Rhipidium* so far described, except *R. parthenosporum*, by the very short branches which bear the sporangia and oogonia. It may be separated from *R. parthenosporum* by the shorter basal portion of the plant, by the presence of zoospores, by the smaller oogonia with papillae on the walls, by the sporangia and oogonia being borne in the same cluster, and by the presence of antheridia. Depauperate forms of *R. americanum* may bear sporangia on short pedicels, but the many plants found on ripe fruits at different times were very constant and apparently developing under suitable conditions and the antheridia were not borne on the stalk bearing the oogonium.

A detailed description of *R. compactum* n. sp. is given below:

Plants appearing on the substratum as small whitish pustules about 0.5–1 mm. in diameter. Individual plants composed of a main trunk, which may or may not be branched, a large number of short secondary branches on which the reproductive organs are borne, and a well developed system of large branched often lobed rhizoids, which may extend into the substratum up to a distance of about 725 μ . Main trunk unbranched or with as many as 8 large branches, 60–83 μ in diameter by 99–214 μ long, constricted slightly at the base where the rhizoids originate. Short secondary branches 9–42 μ (majority about 20 μ) long, from the large trunk, constricted at their point of origin bear the sporangia and oogonia, usually singly, occasionally two, very rarely three. Sporangia 2–10 on a main branch, very variable in shape on the same plant, globose to pyriform ones 33–36 x 42 μ , cylindrical ones 20–29 x 49–70 μ . Zoospores (rarely produced in the laboratory) reniform, biciliate, 6.4–8 x 11.2–12.8 μ , monoplanetic. Oogonia borne on same plant as sporangia and mixed with them, at times even arising from same short branch that bears a sporangium, spherical, 26–40 μ in diameter, wall thin, smooth or usually with minute papillae. Oospores one to an oogonium, 29–33 μ in diameter, wall at maturity sculptured and about 6.6 μ thick. Antheridia one to each oogonium forming a tube to the oosphere and borne on a long antheridial stalk arising from same plant but not from branch bearing the oogonium or in some cases perhaps from a separate plant.

On huckleberry and *Amelanchier* fruits in Mountain Lake at Mountain Lake, Giles County, Virginia, July and August 1936.

PLATE 25

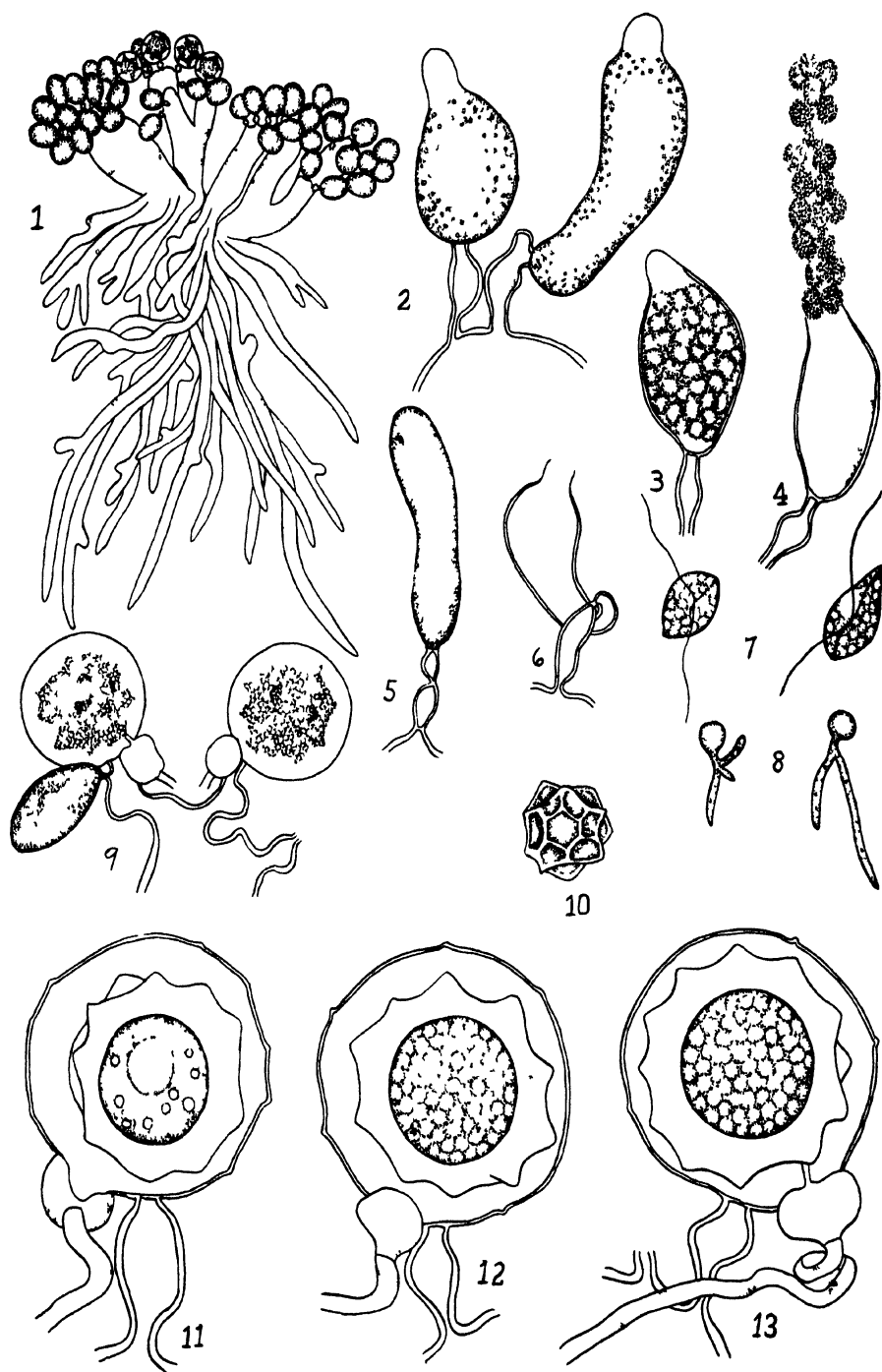
Fig. 1. Cluster of plants showing habit of growth. $\times 111$.

Fig. 2. Two young sporangia with large central vacuoles. $\times 460$.

- Fig. 3. Sporangium with zoospore origins. $\times 460$.
Fig. 4. Sporangium with zoospores escaping. $\times 460$.
Fig. 5. Young cylindrical sporangium borne on a branch constricted in the center. $\times 460$.
Fig. 6. Empty irregular sporangium. $\times 460$.
Fig. 7. Zoospores. $\times 1013$.
Fig. 8. Germinating zoospores. $\times 460$.
Fig. 9. Branch with a young sporangium and young oogonia. $\times 460$.
Fig. 10. Mature oogonium. $\times 1013$.
Fig. 11. Mature oospore. $\times 460$.
Figs. 12-13. Oogonia with antheridia. $\times 1013$.

COKER COLLEGE,
HARTSVILLE, S. C.

PLATE 25



THE GENUS *CYPERUS* IN NORTH CAROLINA

By MILDRED STITES REED

PLATES 26-30

Although there are numerous species of the genus *Cyperus* in North Carolina and individuals of certain species are exceedingly abundant in most parts of the state, there seems to have been no especial work done on the group in this region. The genus has been treated only in general floras, such as Gray's (1908), Small's (1933), and Chapman's (1897), or in works on the *Cyperaceae* in other sections of the country, as Torrey's *Monograph of the North American Cyperaceae* (1836) and Britton's *List of the North American Species of Cyperus* (1886) in which several new forms were described. Little of this work is of recent date and is, therefore, open to certain corrections. Underwood (1932) made a study of the *Cyperaceae* of Tennessee. In 1934 Geise published a paper on the Indiana species of *Cyperus*. There has, however, been no study of the group in North Carolina or in any of the states which are geographically somewhat similar to it.

In view of these facts it has seemed desirable to make a study of the species of *Cyperus* in North Carolina in order to determine the number present, their habitats, the distribution in the state, and, since the genus is taxonomically a difficult one, to attempt to make a simple and usable key to the species.

Although it was impossible for the author to make collections of species of *Cyperus* from all sections, specimens from various herbaria have made it possible to work out fairly satisfactorily the state distribution, for there are representative collections from each of the three main geographical divisions of North Carolina: namely, the coastal plain, the piedmont, and the mountains.¹ As would be expected, the

¹ I wish here to acknowledge the consideration shown by the following herbaria in furnishing their North Carolina specimens of *Cyperus* for study: The United States National Herbarium, the New York Botanical Garden, the Missouri Botanical Garden, the University of North Carolina Herbarium, the University of West Virginia Herbarium, the Academy of Natural Science Herbarium, and the Duke University Herbarium. I wish also to express my appreciation to Dr. H. L. Blomquist, of Duke University, under whose direction this study was carried out.

more typically northern and western species of *Cyperus* are found in the mountainous western part of the state, while extensions of the more characteristically southern forms occur in the southeastern part of the state along the coast. Some species are, however, statewide in distribution.

The genus *Cyperus* belongs to the *Cyperaceae*, or sedge family, which is usually placed with the *Poaceae*, or grass family, in the order *Poales*. The common names used for the genus *Cyperus* are "galingale," "sweet rush," or, most often, merely "sedge." It is one of the largest genera of the *Cyperaceae*, having about 600 species widely distributed but occurring chiefly in the tropics and in the warmer parts of the temperate zones. The total number of species that occur within the United States is about 90, and in the course of this work 32 of these species have been identified as occurring in North Carolina. Among these are three species which apparently have not previously been reported as occurring in North Carolina: namely, *Cyperus Plankii* Britton, collected in Durham and Beaufort counties; *C. Engelmanni* Steud., collected in New Hanover County; and *C. sphacelatus* Rottb., found in "eastern North Carolina" apparently near Wilmington and probably growing on ballast there.

In studies which deal with the identities of species and their distribution over a statewide area, the worker usually finds that certain species have been reported as occurring in the state, which apparently are not really to be found there. Such has been the case in the course of this work. The confusion seems to be due in some cases to the unfortunate amount of synonymy which is present in this genus and in other cases to the confusion of one species with another that is similar in habit.

The first type of confused identity, that due to excessive and inaccurate synonymy, is well illustrated in the species *Cyperus ferox* L. C. Rich., *C. speciosus* Vahl., and *C. Michauxianus* Schultes. The specimens examined which were labeled as any of these species seemed to possess characteristics of all three, fitting one description as well as another. In fact, there seemed to be no distinguishing character on which even a varietal difference might be established, and the conclusion was reached that all of the North Carolina specimens, at least, belong to one species. This opinion has received confirmation in a recently published article by Fernald and Griscom of the Gray Herbarium (Rhodora, May, 1935), in which the species *C. speciosus*, *C. ferox*, and *C. Michauxianus* are discussed. Their conclusion is as follows:

"After attempting to find any character to distinguish these various plants, we are forced to treat them as a single wide-ranging species of which *Cyperus ferax* is the earliest name."

An excellent example of the other type of confused identity, that of mistaking one species for another that is similar in habit, was found in the case of *Cyperus rivularis* Kunth. and *C. diandrus* Torr. Both these species have frequently been identified as occurring in this state. The names seem to have been applied indiscriminately, for upon close examination there seemed to be no constant difference between plants labeled *Cyperus rivularis* and those labeled *C. diandrus*, except that the latter were usually larger than the former. Examination of specimens collected in other parts of the United States, however, showed that there is a great difference between the two. Both species have scales which at maturity are characteristically marked with red, but the manner in which they are marked seems to be distinct for each group. *Cyperus diandrus* has extremely thin, almost transparent scales, in which the red color seems to be concentrated at the margins, leaving the central portion of each side of the scale colorless. Those of *C. rivularis*, on the other hand, are firm and glossy and their color is spread over the entire scale or concentrated toward the center of each side of the scale rather than at its margins. In *C. diandrus* the style branches of every floret are exerted for two millimeters or more of their length, giving the spikelet as a whole a hairy or roughened appearance. *Cyperus rivularis*, however, has many of its styles included, although some of them may be exerted for a considerable distance. But there is no hairy appearance to the spikelet, and usually not even half of the scales have style branches extending conspicuously beyond them. In most taxonomic keys this difference in the length of the style branches is made the specific distinction between the two. When, however, *C. rivularis* is the only species present, as seems to be the case in North Carolina, difficulties arise because certain individuals of this one species seem to vary considerably in the length of the exposed style, and not having a specimen of the true *C. diandrus* for comparison, it is easy to see how a specimen with a few relatively long style branches might be classed as *C. diandrus* and another individual of the same species with shorter styles might be called *C. rivularis*. The evidence, from all the specimens examined in this study, indicates, therefore, that only *C. rivularis* is to be found here along with a more or less well-marked variety, *C. rivularis* var. *elongatus*.

Various species of the genus *Cyperus* appear in a number of types of habitats, ranging from extremely wet situations along lake shores or

in swamps where the lower part of the plant may even be immersed in water, to extremely dry sites in sand along the coastal plain or on xeric hillsides in the central or western parts of the state. There are, of course, all types of variation between these two extremes, and some species seem to be so adapted that they can live in any or all of these habitats. Most species of *Cyperus* seem to thrive on sandy and often acid soil. The majority are found in open places where there is an abundance of light, but several species are frequently found in woodlands or shaded situations.

The plants in the genus *Cyperus* are either annual or perennial. The roots are fibrous and in some cases have small, tuberlike swellings, the "tubers" being edible. The culms are triangular, erect or merely ascending, simple, and, in the case of perennial species, their bases are hardened into corms. Most of the perennials are also rhizomatous. The plants may be either solitary or tufted. Leaves are basal with linear-lanceolate blades, usually elongated but rarely reduced to membranaceous sheaths at the base of the stem. The inflorescence is a terminal spicate umbel, subtended by an involucre of from one to several, usually elongated, leafy bracts. The inflorescence may be either simple or compound, and the rays of the involucre, when present, are sheathed at the base and unequal in length. The spikelets are few to many-flowered, usually elongated, borne in spikes, panicles, clusters, or heads, and are flat or almost terete. Scales of the spikelets are two-ranked, conduplicate, and keeled. They may be either deciduous from the rachilla or persistent until after it has fallen from the rachis at maturity. The rachilla is often winged. The flowers are perfect and are without a perianth or perianth bristles. The stamens are from one to three in number. The ovary is superior, and its style may be either two- or three-cleft and is deciduous. If the style is two-cleft, the achene is lenticular; in species in which the style is three-cleft the achene is three-angled. In all cases, however, the achene is without a beak or tubercle.

In this state some species of *Cyperus* begin flowering by the middle of June, and one species, *C. rotundus*, has been collected in flower as late as the first of November. The genus as a whole is, however, a typically late summer plant in time of flowering. Best specimens should, therefore, be collected after this time, as most of the species fruit after the middle of August, and usually the mature fruit is necessary for the correct identification of a species.

The manual most used for identification in the course of this work

and upon which the nomenclature is to a great extent based is Small's *Manual of the Southeastern Flora*. Other manuals used include Gray's *Manual* (ed. 7), Britton and Brown's *Illustrated Flora of the Northern States and Canada* (ed. 2) and Chapman's *Flora of the Southeastern United States* (ed. 3).

The following key is as original and practical as it has been possible to make it, but in some instances the diagnostic distinctions are based on those used in Small's key to the genus *Cyperus*.

For accurate identification of any species of *Cyperus* it is necessary to have a specimen with mature fruits. The following key is based upon the characteristics of mature plants and mature fruits. If an attempt is made to identify an immature specimen, differences in color and length of parts should be taken into consideration, young spikelets and scales usually being green and all parts of the young plants being much smaller than the corresponding parts of a mature specimen.

KEY TO THE SPECIES

1. Achenes lenticular, their styles 2-cleft; plants annual.
2. Inflorescence distinctly terminal; spikelets strongly flattened; achenes laterally compressed.
3. Scales without scarious margins or with very narrow ones along the sides; achenes much shorter than the scales.
4. Spikelets obtuse to acutish, thick and rigid, yellow or red; rachilla wingless or very inconspicuously winged; achenes suborbicular or broadly obovoid.
5. Spikelets yellow or greenish, 1-2.5 mm. wide; mature achenes suborbicular, black, and shining 1. *C. flavescent*
5. Spikelets red or variegated with red, 2.5 mm. or more in width; mature achenes grayish brown, broadly obovoid.
6. Spikelets 5-12 mm. long; scales 2 mm. long 2. *C. rivularis*
6. Spikelets 15-25 mm. long; scales about 2.5 mm. long
2a. *C. rivularis* var. *elongatus*
4. Spikelets sharply acute, brown to greenish brown, not thick; rachilla somewhat winged; achenes linear to narrowly obovoid.
7. Scales 2.5-3 mm. long; achenes light brown, about 1.5 mm. or somewhat less in length 3. *C. filicin*
7. Scales 2 mm. or less in length; achenes dark brown or gray, 1 mm. or less in length.
8. Spikelets 1.5 mm. or more in width; mature achenes dark brown, narrowly obovoid 4. *C. microd*
8. Spikelets about 1 mm. in width; mature achenes dark gray, linear to linear-ellipsoid 5. *C. paniculatus*
3. Scales with broad scarious margins at the tips; achenes as long as the scales 6. *C. sabulos*
2. Inflorescence appearing lateral; spikelets subterete; achenes dorsally compressed 7. *C. laevigatus*

1. Achenes 3-angled, their styles 3-cleft; plants annual or perennial.
9. Spikelets flattened.
 10. Scales 3 mm. or less in length, deciduous from the rachilla at maturity.
 11. Scales ending in long, subulate, recurved awns; achenes wedge-shaped
 8. *C. inflexus*
 11. Scales acute, obtuse, or mucronate, not ending in recurved awns; achenes not wedge-shaped.
 12. Spikelets broadly ovate, in dense, capitate clusters; stamens 1.
 13. Scales nearly straight; achenes 1.5 mm. long, narrowly ellipsoid, about $\frac{1}{2}$ as long as the scales. 9. *C. virens*
 13. Scales curved; achenes 1 mm. long, linear, about as long as the scales
 10. *C. pseudovegetus*
 12. Spikelets linear or ellipsoid, digitate or spicate; stamens more than one.
 14. Spikelets lax in soft, plume-like, elongated spikes; achenes as long as the scales. 11. *C. Iria*
 14. Spikelets rigid in stiff clusters or spikes; achenes much shorter than the scales.
 15. Spikelets in digitate clusters.
 16. Spikelets green or yellow; achenes 1 mm. or more in length
 12. *C. compressus*
 16. Spikelets red or variegated with red; achenes less than 1 mm. long.
 17. Culms weak; leaves usually reduced to sheathing scales at the base of the culm; spikelets less than 1 mm. wide. 13. *C. Haspan*
 17. Culms rigid; leaves with elongated blade; spikelets 1.5 mm. or more in width.
 18. Spikelets 4-15 mm. long; 3-4 mm. wide; scale tips not closely appressed, strongly mucronate. 14. *C. dentatus*
 18. Spikelets 8-20 mm. long, 2-2.5 mm. wide; scale tips more closely appressed and not so strongly mucronate as in the species
 - 14a. *C. dentatus* var. *stenostachys*
 15. Spikelets in definite stiff spikes.
 19. Spikelets red or variegated with red.
 20. Spikelets less than 1 mm. wide; scales distant, truncate at the tips
 15. *C. distans*
 20. Spikelets 1.5 mm. or more in width; scales imbricated, obtuse or acute.
 21. Scales with very broad green keels, their sides green or yellow and variegated with red. 16. *C. sphacelatus*
 21. Scales with narrow, bright green keels; their sides dark purple-red. 17. *C. rotundus*
 19. Spikelets yellow, brown, or chestnut.
 22. Spikelets few, distant in broad, stiff spikes; scales 2-3 mm. long; achenes brown.
 23. Spikelets 2-3 mm. wide. 18. *C. esculentus*
 23. Spikelets 1 mm. or less in width
 - 18a. *C. esculentus* var. *angustispicatus*
 22. Spikelets many in dense, cylindric spikes; 1 mm. or less in width; scales 1-1.5 mm. long; achenes pale. 19. *C. erythrorhizos*
 10. Scales 4-5 mm. long; either the scales deciduous from the rachilla at maturity, or the rachilla deciduous from the rachis, or both deciduous.

24. Umbel simple or compound; spikelets 8 mm. or more in length; 7 or more flowers on a spikelet.
25. Scales closely appressed; sheaths of the rays deeply 2-toothed.
26. Spikelets 8-18 mm. long; 7-12-flowered.
27. Rays 2-10 cm. long..... 20. *C. strigosus*
27. Rays 20-30 cm. long..... 20a. *C. strigosus* var. *elongatus*
26. Spikelets 20-30 mm. long, 10-15-flowered
- 20b. *C. strigosus* var. *robustior*
25. Scales lax and spreading at maturity; sheaths of the rays obscurely 2-toothed or truncate..... 20c. *C. strigosus* var. *stenolepis*
24. Umbel very compound; spikelets 6-13 mm. long, 4-6-flowered
- 20d. *C. strigosus* var. *compositus*
9. Spikelets not flattened, terete or subterete; rachilla deciduous from the rachis at maturity, the scales persistent on the rachilla.
28. Scales distinctly imbricated; spikelets deciduous as a whole above the lowest pair of scales.
29. Spikelets about 1 mm. wide or less; subulate to linear-subulate; achenes 2.5 or more times as long as broad, linear to linear-ellipsoid.
30. Spikelets subulate; scales closely appressed and imbricated.
31. Spikelets distant in loose, elongated spikes..... 21. *C. refractus*
31. Spikelets crowded into short-capitate or narrowly cylindric heads.
32. Heads obovoid or oval; at least some of the spikelets reflexed.
33. Culms rough, at least near the top; heads strongly obovoid; practically all the spikelets reflexed.
34. Rays rough; involucre bracts shorter than the umbel; heads greenish, short-turbinate..... 22. *C. retrofractus*
34. Rays smooth; the longer involucre bracts surpassing the umbel; heads golden-brown at maturity, elongated obovate-cylindric
23. *C. dipsaciformis*
33. Culms smooth; heads oval; usually only the lower spikelets reflexed..... 24. *C. lancastricensis*
32. Heads cylindric or globose.
35. Heads cylindric.
36. Sheaths of the rays distinctly 2-toothed; spikelets 2-4 mm. long, 1-2-flowered..... 25. *C. Torreyi*
36. Sheaths of the rays truncate or mucronate; spikelets 5-7 mm. long, 3-5-flowered..... 26. *C. Plankii*
35. Heads globose..... 27. *C. ovularis*
30. Spikelets linear or linear-subulate; scales rather loosely appressed, somewhat imbricated; heads globose..... 28. *C. globulosus*
29. Spikelets over 1.5 mm. wide, linear to linear-ellipsoid; achenes not more than twice as long as broad, broadly elliptic.
37. Culms slightly roughened on the angles near the top, rigid; spikelets in loose, cylindric spikes; rachilla broadly winged..... 29. *C. tetragonus*
37. Culms smooth, filiform, sometimes lax; spikelets in globose, capitate clusters; rachilla narrowly winged or wingless..... 30. *C. filiculmis*
28. Scales distant or very slightly imbricated; spikelets breaking up into 1-fruited joints at maturity.

38. Scales of the spikelet remote, their tips distant from the bases of those above them on the same side by about half the length of the scale; achenes narrowly linear-ellipsoid.....31. *C. Engelmanni*
38. Scales of the spikelets only slightly distant from those above them or somewhat imbricated; achenes ellipsoid or ellipsoid-obovoid...32. *C. serax*

LIST OF SPECIES WITH HABITATS AND DISTRIBUTION

1. *Cyperus flavesces* L. Sp. Pl. p. 46. 1753. (Fig. 4)
Damp, usually sandy soils, in low grounds, sun or shade. Widely distributed in western and central sections and to some extent in the southeastern part of the state. July-October.
2. *Cyperus rivularis* Kunth. Enum. Pl. ii:6. 1837. (Fig. 10)
Wet or moist, usually sandy soils. Occurring in practically all sections of the state. August-November.
- 2a. *Cyperus rivularis* Kunth. var. *elongatus* Boekl. Linnaea xxxvi. 1870. (Fig. 9)
Habitat and range with the species. August-November.
3. *Cyperus filicinus* Vahl. Enum. ii:332. 1806. (Fig. 3)
On or very near the seashore, on sand or in brackish marshes. Eastern North Carolina. August-October.
4. *Cyperus microdontus* Torr. Ann. Lyc. N. Y. iii:225. 1836. (Fig. 5)
Wet or moist, sandy soils. Widely distributed in the eastern and central parts of the state. August-November.
5. *Cyperus paniculatus* Rottb. Descrip. & Icon. p. 40. 1773. (Fig. 6)
(*C. Gatesii* Torr.)
Low, wet ground near the coast. Found only in the southeastern part of the state. Rare. August-October.
6. *Cyperus sabulosus* Mart. and Schrad. Mart. Fl. Bras. 21: 10. 1842. (Fig. 1)
(*C. flavicomus* Michx.)
Damp to fairly dry, usually sandy soil. Common on cultivated ground. One of the most common species in the central part of the state. July-November.
7. *Cyperus laevigatus* L. Mant. ii: 179. 1771. (Fig. 2)
Sandy soil near the seashore. Found only in the southeastern portion of the state. September-October.
8. *Cyperus inflexus* Muhl. Desc. Gram. p. 16. 1817. (Fig. 14)
Damp or wet, sandy soil, often on lawns or cultivated ground. Found in the central portion of the state. July-October.
9. *Cyperus virens* Michx. Fl. Bor. Amer. 1: 28. 1803. (Fig. 11)
Sandy soil, moist to fairly dry sites. Found only on the coastal plain in the southeastern part of the state. Rather rare. July-October.
10. *Cyperus pseudovegetus* Steud. Syn. Pl. Cyp. p. 24. 1855. (Fig. 12)
(*C. calcaratus* Nees.)
Wet, sandy soils in marshes or on river banks. Common in the eastern and central parts of the state.
11. *Cyperus Iria* L. Sp. Pl. p. 45. 1753. (Fig. 20)
Low, wet ground or marshes, particularly on cultivated ground. Found in the central part of the state. September-November.

12. *Cyperus compressus* L. Sp. Pl. p. 46. 1753. (Fig. 13)
Moist to dry, usually sandy soil. A common weed on lawns and cultivated ground. Found in the central and eastern section of the state.
July–November.
13. *Cyperus Haspan* L. Sp. Pl. p. 45. 1753. (Fig. 23)
Very damp or wet soils in swamps or wet woodlands. Found in the eastern and east central parts of the state.
July–October.
14. *Cyperus dentatus* Torr. Fl. N. and Mid. U. S. 1: 61. 1824. (Fig. 18)
Moist to fairly dry, sandy soils. Found only in the southeastern part of the state. Not common.
August–October.
- 14a. *Cyperus dentatus* Torr. var. *clenostachys* Fernald. Rhodora viii: 126. 1906. (Fig. 19)
Usually found associated with the species. August–October.
15. *Cyperus distans* L. f. Suppl. p. 103. (Fig. 21)
Wet woods or swamps in the southeastern part of the state.
July–September.
16. *Cyperus sphacelatus* Rottb. Descr. Nov. Pl. 26: Prog. 21. 1778. (Fig. 15)
Found in the southeastern part of the state along the coast where it was probably growing on ballast. Extremely rare. Only one specimen has been collected, but it is unmistakable.
August–September.
17. *Cyperus rotundus* L. Sp. Pl. p. 45. 1753. (Fig. 29)
(*C. hydra* Michx.)
Moist to fairly dry habitats. A common weed on lawns. Found in the central portion of the state.
July–November.
18. *Cyperus esculentus* L. Sp. Pl. p. 45. 1753. (Fig. 26)
(*C. phymatoides* Muhl.)
Moist soils, usually in open fields. Often a troublesome weed in cultivated ground. Occasionally cultivated as a stock food because of its edible "tubers." Statewide in distribution.
July–October.
- 18a. *Cyperus esculentus* L. var. *angustispicatus* Britton. Bull. Torr. Bot. Club 13: 211. 1886.
Its range and habitats are those of the species. July–October.
19. *Cyperus erythrorhizos* Muhl. Descr. Gram. p. 20. 1817. (Fig. 27)
Damp, sandy soils along river banks or in marshes. Found in the central and southeastern portions of the state.
August–October.
20. *Cyperus strigosus* L. Sp. Pl. p. 47. 1753. (Fig. 30)
Moist to dry soils. Common in cultivated land. Widely distributed in all parts of the state.
July–October.
- 20a. *Cyperus strigosus* L. var. *elongatus* (Torr.) Britton. Bull. Torr. Bot. Club 13: 212. 1886.
Found in the central part of the state in habitats similar to those of the species. July–October.
- 20b. *Cyperus strigosus* L. var. *robustior* Kunth. Enum. ii: 88. 1837. (Fig. 7)
Its range and habitats are with those of the species. July–October.
- 20c. *Cyperus strigosus* L. var. *stenolepis* (Torr.) Kukenthal. Fedde, rep. Spec. Nov. xxiii: 189. 1926. (Fig. 16)
This variety is usually found in swamps or low grounds but sometimes

occurs on drier sites. (The reduction of this usually recognized species to the rank of a variety is based upon an examination of the type specimen (Curtis) at the Herbarium of the New York Botanical Garden.)

July-October.

- 20d. *Cyperus strigosus* L. var. *compositus* Britton. Bull. Torr. Bot. Club xiii: 212. 1886. (Fig. 8)

Distribution and habitats similar to those of the species. July-October.

21. *Cyperus refractus* Engelmann ex Boekl. Linnaea xxxvi: 369. 1869-1870. (Fig. 24)

Fairly moist to dry soils, in sun or shade, usually in woods or dry fields. Found mostly in the western and west central portions of the state.

July-September.

22. *Cyperus retrofractus* (L.) Torr. A. Gray Man. p. 519. 1848. (Fig. 25)
Dry, usually sandy soils, in woods or fields. Statewide in distribution.

July-October.

23. *Cyperus dipsaciformis* Fernald. Rhodora viii: 127. 1906. (Fig. 28)
Dry soils, usually in shady sites. Statewide in distribution.

June-October.

24. *Cyperus lancastriensis* Porter. A. Gray Man. p. 555. 1848. (Fig. 22)
Damp to dry soils, particularly in open fields. Found in the central and western parts of the state. July-September.

25. *Cyperus Torreyi* Britton. Bull. Torr. Bot. Club vii: 48. 1880. (Fig. 17)
(*C. cylindricus* (Ell.) Britton.)
Moist to fairly dry, sandy soils. Found in all parts of the state.

June-September.

26. *Cyperus Plankii* Britton. Small's Fl. S.E.U. S. p. 172. 1913. (Fig. 31)
Moist soils, particularly in open fields. Collected in the central and eastern parts of the state. July-October.

27. *Cyperus ovularis* (Michx.) Torr. Ann. Lyc. N. Y. iii: 278. 1836. (Fig. 32)
Moist to dry habitats, in fields or on stream banks. Common on cultivated ground. Widely distributed in the central and western parts of the state. Rather rare in the east. June-October.

28. *Cyperus globulosus* Aubl. Pl. Guian. i: 47. 1775. (Fig. 35)
(*C. echinatus* (Ell.) Wood., *C. Baldwinii* Torr.)

Rather dry, sandy woods or fields. Collected in eastern and central portions of the state and found as far west as Rowan County. July-October.

29. *Cyperus tetragonus* Ell. Sketch i: 71. 1821. (Fig. 33)
Sandy soils, often in woodlands. Found only in the southeastern part of the state along the coast. July-October.

30. *Cyperus filiculmis* Vahl. Enum. ii: 328. 1806. (Fig. 36)
Dry, usually sandy soils. Statewide in distribution. June-October.

31. *Cyperus Engelmanni* Steud. Syn. Pl. Cyp. p. 47. 1885. (Fig. 34)
Collected in the eastern part of the state. Probably adventive. Rare. August-October.

32. *Cyperus ferax* L. C. Rich. Act. Soc. Hist. Nat. i: 106. 1792. (Fig. 37)
(*C. speciosus* Vahl., *C. Michauxianus* Schultes.)
Moist, usually sandy soils in swamps and along the streams and lakes. Found only in the eastern part of the state. August-October.

EXCLUDED SPECIES

The following species have been reported by collectors and writers from various parts of the state, but because they have not been observed in any of the collections studied, the existing data have not been considered sufficient to validate their inclusion in this paper.

Cyperus cayennensis (Lamarck.) Britton., reported from Currituck County by McAtee.

Cyperus repens Elliott, reported by Torrey as being collected by Muhlenberg in North Carolina.

Cyperus leptos Schultes, reported by Torrey as being collected by Curtis in New Hanover County.

Cyperus hystrixinus Fernald, reported by McAtee from Currituck County.

Cyperus diandrus Torr., reported by various collectors from North Carolina but not observed among the specimens studied.

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PLATE 26

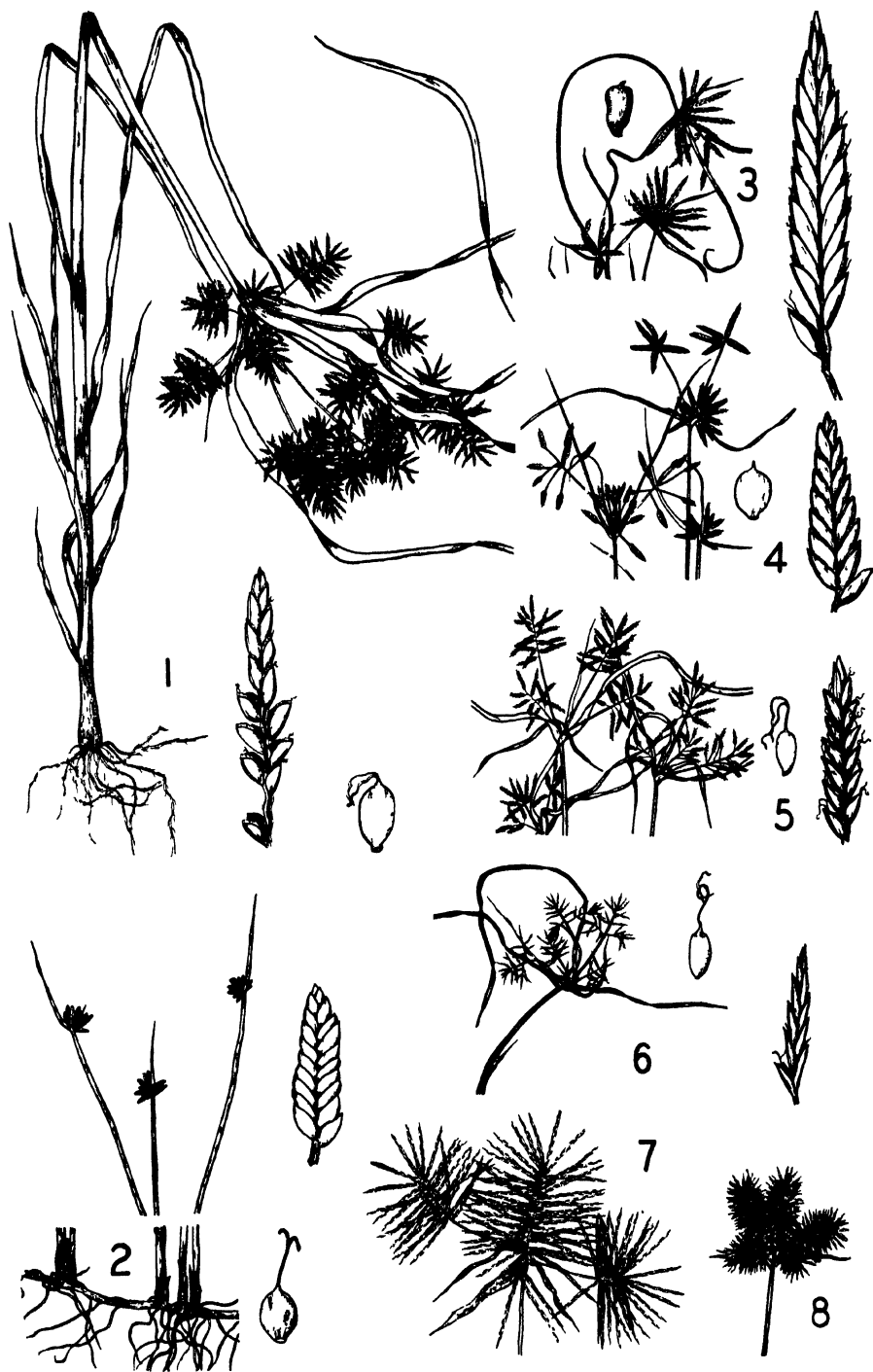


PLATE 27

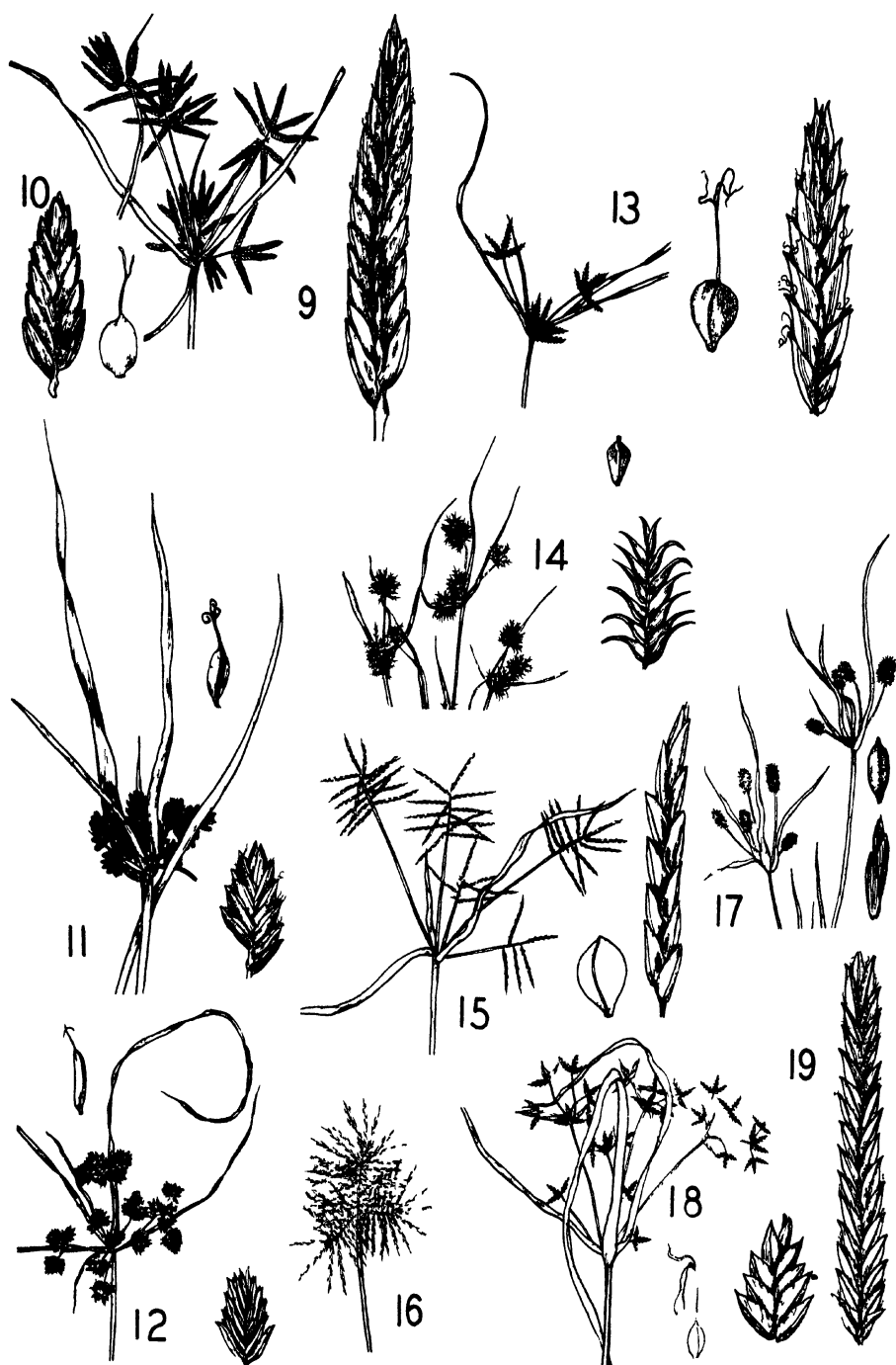


PLATE 28

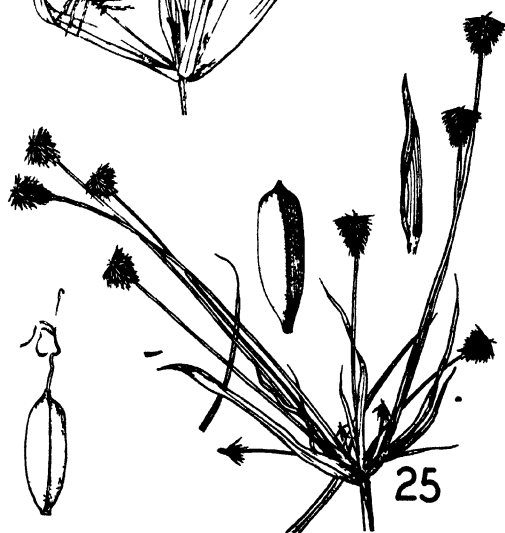
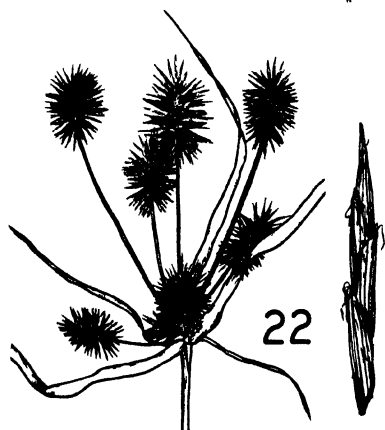
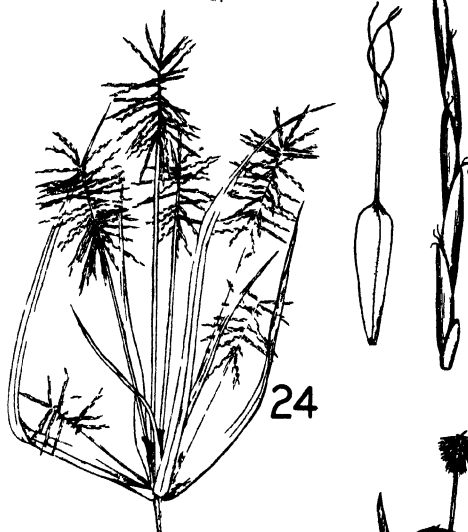
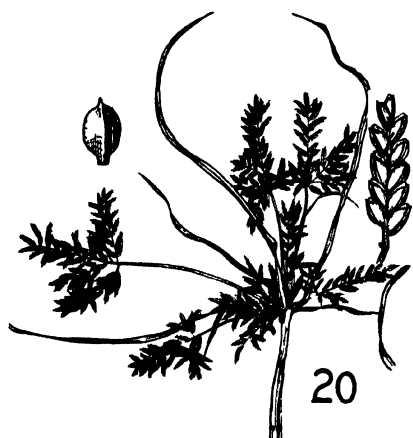


PLATE 29

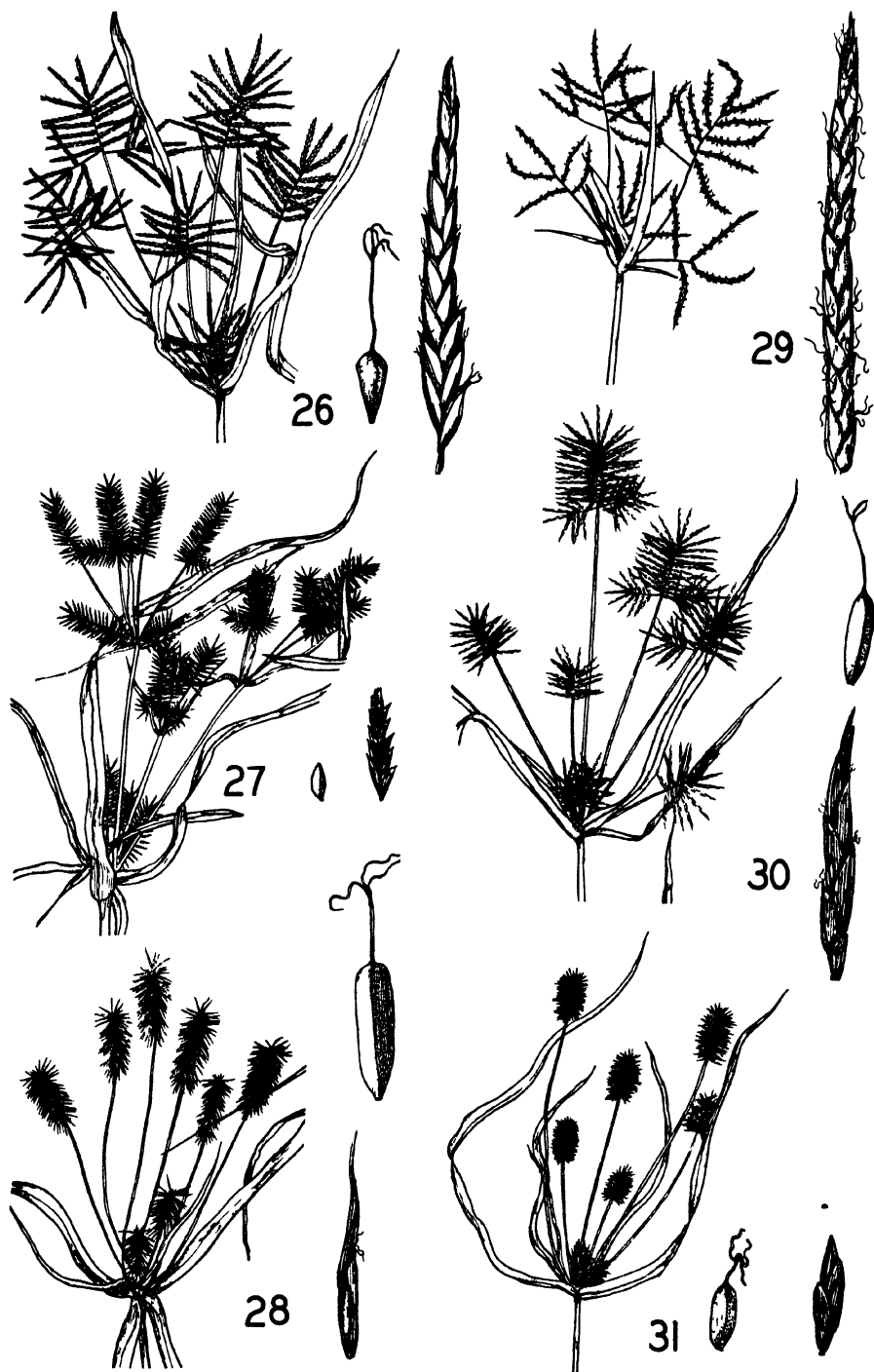
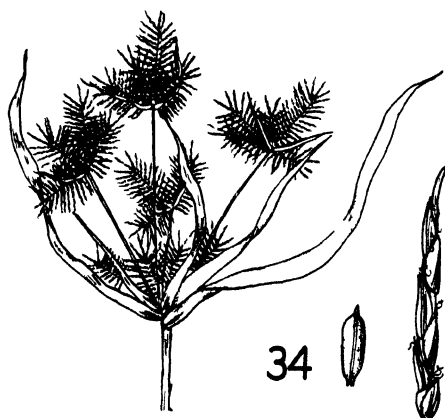
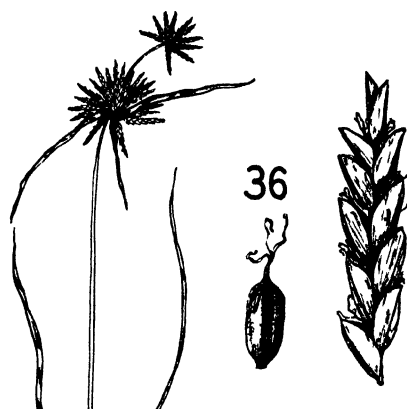


PLATE 30



SPERMATOGENESIS IN MARSILEA

By A. G. LANG

PLATES 31-35

INTRODUCTION

Although many detailed studies of spermatogenesis in ferns have been made during the last forty years, most of these investigations have been concerned primarily with the problem of centrosomes and blepharoplasts and until recently no serious efforts were made to interpret the structure of the mature spermatozooids. In 1897 Belajeff (5) announced the presence of a deeply-staining band in the fern spermatozoid and believed this structure to give rise to the flagella in the same way that the blepharoplast of the gymnosperm spermatozooids gives rise to flagella. This interpretation was widely accepted until Mühldorf (28) pointed out in 1930 that the flagella of the typical fern spermatozoid are not attached to the blepharoplast structure but are outgrowths of a plasma band located between the blepharoplast and the nuclear portion of the spermatozoid. Miss Rogers had arrived at essentially similar conclusions four years earlier (31). Shortly after the paper by Mühldorf had appeared, Miss Dracinschi published the results of her investigations (14, 15, 16). She confirmed the observations of Mühldorf and indicated further that a basal granule is found at the insertion point of each flagellum. Miss Dracinschi also believed the blepharoplast structure to be composed of chondriosomal material. Miss Rogers (31) had described similar basal granules sometime previously and Motte (26) had believed the blepharoplasts of the bryophytes and the ferns to be chondriosomal substance. More recently, Yuasa has investigated the morphology of various fern spermatozooids and has largely confirmed the findings of Miss Dracinschi concerning the structural organization of these bodies (46, 47, 48, 50, 51).

Although certain other recent publications, notably those of Weier (41) and Miss Rankin (30), contributed toward the formulation of the present investigation, these need not be discussed here since the following report mainly concerns the structure of the mature spermatozoid. However, the present study was undertaken to trace the occurrence and behavior of all noteworthy cellular constituents during spermato-

genesis and to ascertain more precisely the structure of the *Marsilea* spermatozoid in relation to the reports of Miss Dracinschi, Yuasa, Motte, and Miss Rogers.

MATERIALS AND METHODS

Sporocarps of *Marsilea quadrifolia* were obtained through the kindness of Dr. E. L. Stover at the Eastern Illinois State Teacher's College. Dr. H. C. Aase of the State College of Washington collected sporocarps of *M. vestita*, but unfortunately these were not viable.

For the study of the mature spermatozoid, sporocarps were cut open with a razor blade and placed individually into beakers of water on a hot plate at 28°C. The spermatozooids seem to thrive equally well in distilled water (pH about 5.7) and in tap water (pH about 9 to 10 in Raleigh and about 7.2 in Chapel Hill). At 28 to 30°C., swimming spermatozooids may be obtained regularly in about 6½ hours. They are most abundant usually from 1 to 4 hours after the first dehiscence and the collections for study can be made most profitably about 3 hours after the first swimming spermatozooids are seen. About 2 hours after the first antheridial dehiscence, the inner channel of the mucilaginous envelope becomes suddenly filled with many spermatozooids. Most of these are deprived of their normal vesicle and bear instead a larger and much more hyaline secondary vesicle-like membrane which is sometimes difficult to demonstrate. Embedded in the mucilaginous envelope itself at this time there are found many extended spirals which are especially favorable for study. Material germinated at temperatures lower than 28°C. has shown no differences in any structural detail.

Active spermatozooids were fixed with osmium fumes and stained according to the technics described by Steil (36, 38), Mühldorf (27, 28, 29), Dracinschi (14, 15, 16), and particularly those by Yuasa (47). The most useful preparations of this type were obtained with 1% solutions of iodine green, basic fuchsin, carbol fuchsin, safranin O, and neutral red, but other stains such as acid fuchsin, methyl green, crystal violet, anilin blue, Delafield's and Heidenhain's hematoxylin were also used. Heitz's hot aceto-carmine was most useful when used on material treated for 2 to 5 minutes with strong osmium fumes.

Spermatozooids were also studied from paraffin sections. This material and material of the developmental stages were fixed in the following solutions which were found most useful in the identification and fixation of specific constituents of the spermatozooids and cells of the developmental stages:

1. Sharp's *Marsilea* formula (33).
2. Nawaschin (Craf's modification).
3. Nawaschin (author's modification):

Chromium trioxide.....	1 gram	}	13 cc.
A. Acetic acid.....	7 cc.		
Distilled water.....	92 cc.		
B. 4% Formalin.....			13 cc.
C. 1% Osmium tetroxide.....			1 cc.
4. Regaud's method, in various forms of post-fixation.
5. Lewitsky's formula (25), used with formalin "neutralized" with CaCO_3 , or with MgCO_3 , or used with acid formalin.
6. Lewitsky's formula (author's modification):

A. 1% Chromium trioxide.....	14 cc.
B. 10% Formalin.....	14 cc.
C. 1% Osmium tetroxide.....	0.8 cc.
7. Champy (7-7-4 formula).

Spermatozooids fixed in these and other solutions were stained with various dyes already mentioned. Basic fuchsin, safranin O, iodine-crystal violet, and Heidenhain's hematoxylin were most useful. Formulae 1, 2, 3, 5, and 6 gave best results in the developmental stages of the microspore and Regaud's formula was especially excellent during late spermatoteleosis. Material was washed in tap or distilled water for varying periods of time according to the fixative used and was dehydrated in N-butanol in about 18 steps or it was infiltrated by means of petroleum ether. Acetic acid mordants were especially valuable for hematoxylin staining (23) and the degree of destaining was varied to reduce the probabilities of misinterpretation.

DESCRIPTION

The divisions of the microspore. I have satisfied myself that the cellular development in the microspore occurs precisely as described by Sharp (33). A more detailed analysis of the entire developmental history has not been completed and will not be reported here.

Spermatoteleosis. Being interested largely in the relation between centrosomes and blepharoplasts, Sharp did not attempt to follow the later stages in spermatoteleosis and probably for this reason his description for this phase of development does not agree in all details with my observations.

The first of these details concerns the maturation of the blepharoplast. Following the use of the fixative which Sharp employed, one would clearly be inclined to agree with him that the blepharoplast elongates in the cytoplasm of the spermatid and that during this elon-

gation the blepharoplast extends forward in advance of the metamorphosing nucleus. However, if we study preparations of the Lewitsky or Nawaschin type, we would be inclined to interpret the relation of the blepharoplast to the nucleus somewhat differently. Such preparations would lead one to the interpretation that the blepharoplast, almost from the first phases of its elongation, is located within the nuclear membrane and that it never extends forward beyond this membrane. The blepharoplast elongation would appear, not merely to precede the nuclear elongation, but to be a mechanical agency which is responsible for the elongational distortions of the nucleus. The blepharoplast seems to force the nuclear membrane into a condition of apparent stress and seems to be the cause of the membrane distortions which result in the eventual spiral form. During this reorganization of the spermatid materials, the anterior and growing end of the blepharoplast appears to be invested with a very close-fitting portion of the nuclear membrane. The forward part of this membrane at this stage is apparently entirely devoid of structurally organized contents and could not be seen in preparations where fixatives of the "nuclear" type had been used. Following fixations which are more favorable for the preservation of the cytoplasm, however, the karyolymph contents of this membrane can be seen as a transparent space which is definitely delimited from the dark cytoplasmic background. This nuclear portion is very thin at the extreme anterior end even at maturity. It would seem probable, therefore, that the blepharoplast becomes intra-nuclear at an early stage and that it remains within the nuclear membrane thereafter. Further evidence in favor of this interpretation will be brought forward later.

During the early metamorphosis of the nucleus, the chromatin is organized very much as shown by Sharp (33, Figure 37). This material soon clearly resolves itself into four definite chromonemata which as almost parallel strands make their way forward into the anterior portions of the spiral nuclear membrane. Eventually they are spacially arranged as four parallel chromonemata which extend from the posterior end of the spermatozoid well forward into the anterior end. This chromatin behavior can be followed clearly in preparations stained with iodine-crystal violet following Sharp's fixative. "

Concurrently with this nuclear reorganization, the cytoplasm undergoes a remarkable series of changes. Even before the nucleus and the blepharoplast have formed one complete spiral, the distending nuclear portion already lies in contact with the plasma membrane at the periphery of the spermatid. The blepharoplast is at the outer limit of the

nuclear spiral. In the central part of the spermatid, from which the nucleus is being withdrawn, there appear, in contact with the nuclear membrane, a number of large, clear spaces which are interpreted as vacuoles. As the nucleus becomes more completely retired from this central portion of the spermatid, the vacuolated region increases in size and certain materials from the cytoplasm move into this formative vesicle. A number of small but conspicuous starch grains develop here very soon after the vesicle first becomes delimited as a vacuolated region. The large starch grains in the peripheral parts of the microspore concurrently undergo a partial digestion. When the development of the spermatid is proceeding at a temperature of 30°C., the first starch appears within the newly-formed vesicle about fifty minutes after the blepharoplast begins to elongate. The mature vesicle contains within its membrane a loose network of cytoplasmic strands in which are suspended a number of plastid primordia. Some of these plastid bodies remain free of starch. A ribbon-like band of substance of an undetermined nature is also found in the mature vesicle, its component substance having been derived from many scattered, irregular-shaped, and chromatic masses of material which are found in the cytoplasm of all spermatogenous cells. This material is presumably comparable to the heavily-staining material found in the vesicles of various bryophyte spermatozoids by Steil (37).

The cytoplasm at the posterior end of the spermatid becomes progressively thinner as the vesicle increases in volume. The cytoplasm migrates into the anterior half of the spermatid and forms a long, cytoplasmic spiral which is at first thickest at its anterior end. The outer and peripheral side of this cytoplasmic spiral is continuously concave throughout its length. The developing nuclear spiral lies within this concavity and during its development always moves forward in this cytoplasmic channel. When the nuclear coil has become almost complete, the cytoplasmic spiral withdraws slowly to the anterior end where it forms a semi-cylindrical, compact block of cytoplasm which partially surrounds the anterior, free coils of the spermatozoid. In this anterior mass of cytoplasm, by Regaud's technic, are seen several very distinct and intensely chromatic bodies. Sometimes all of this material is found in a single conspicuous spherical body (Figure 16). This is apparently the "Nebenkern" which Yamanouchi described for *Nephrodium* (44).

Cilia can be found growing from the region of the nucleus long before the spiral body is complete. They are entirely unstained when hematoxylin is used. At first they are very thick and short, but as they

grow in length they become very slender. The precise point from which the cilia first develop has not been determined. When fully grown, the cilia are probably less than .1 micron in diameter and bear "end pieces" which are about 4 microns long. The length of the flagella could not be determined accurately because the flagellar filament becomes coiled into a regular spiral which gives one an erroneous impression that the flagella are approximately .18 of a micron wide.

Shortly before antheridial dehiscence, the spermatozooids are symmetrically arranged within each antheridium in two groups of four. The cytoplasmic cap is left behind when the spermatozooids are liberated.

Various other less obvious features of spermatotelyosis will not be described at this time.

The mature spermatozoid. The general appearance of the spermatozoid is indicated by figures 1, 2, 3, 4, 5, 17, 18, 19.

The blepharoplasts. In paraffin material, the most conspicuous structural elements of the spermatozoid are the blepharoplasts. These extend from the anterior tip of the spermatozoid to the posterior end, but occasionally the nuclear body extends several microns beyond the blepharoplasts at the posterior end.

The blepharoplasts are very obviously two parallel strands rather than a single unit (Figure 9). These two blepharoplasts are morphologically identical. Near the anterior end there are frequently seen very definite clear areas in these structures. These hyaline segments are always of uniform size and appearance (Figure 25). They were ignored at first on the assumption that they result from the sectioning process, but that this is not the case can be readily established by means of focusing alone. Further evidence that they are characteristic of the blepharoplasts is found in their constancy of position. In one instance a similar clear segment was seen in only one of the two blepharoplasts. Each blepharoplast is at its widest point about .3 to .4 of a micron in diameter and in its anterior end the diameter is apparently less than one-fourth as great.

These blepharoplasts give some evidence that they have an internal structure. When hot aceto-carmin is applied to the spermatozooids which have been treated with osmium fumes for about five minutes, one can detect a very minute and preferentially-stained coil which seems to be located within a blepharoplast or intimately near it. Only by very delicate focusing can one ascertain the coiled nature of this chromatic substance. This coil is extremely uniform in its windings, but its

coiled nature could be established only in the thicker portions of the blepharoplasts. The substance composing this coil, however, extends over the entire length of the spermatozoid. The aceto-carminic soon becomes general throughout the blepharoplasts and these details become obscure. The blepharoplasts, however, are then revealed as two very obviously separate strands (Figure 9). Material fixed with Lewitsky's solution and stained with Heidenhain's hematoxylin will, under conditions of strong destaining, also indicate the existence of a blepharoplast coil.

In the light of these observations it is interesting but perplexing to consider certain results with vital dyes. Neutral red (1:20,000), when applied to living spermatozooids, will stain the peripheral parts of the blepharoplasts and give these structures the appearance of two hollow tubes. Methyl green, similarly applied, will stain the blepharoplasts in the same manner but less conspicuously. Janus green, under the same conditions, will, in time, stain the parallel blepharoplasts as a single and very slender filament.

These observations would seem to indicate that the blepharoplasts may be two-component structures, both components being essentially similar substance, the one being organized as a matrical system about the other very much as chromosome matrices may enclose within them the chromonemata. This interpretation of an internal structural differentiation in the blepharoplasts, however, needs to be verified in more favorable material.

The spermatozoid body. The body portion of the spermatozoid consists primarily of karyolymph. This part of the spermatozoid is characteristically circular in cross-section, but in many cases the body might become greatly distorted. If we bring into consideration the effects of the various microchemical reagents, we find that the body, at its widest point, is usually about 1.7 microns in diameter. At the anterior end it is approximately one-half micron in diameter. The entire spermatozoid in the swimming condition is a spiral consisting of about seven to nine turns. The blepharoplasts are always seen at the extreme inside of this spiral, but in partially extended spirals the blepharoplasts are usually found at the upper and forward side of the body as shown by figures 10, 11, 13, 19, and by Sharp (33, Figure 42). When fully extended the spermatozoid body is approximately 110 to 120 microns long.

Paraffin material stained with Heidenhain's hematoxylin following Nawaschin and Lewitsky fixations affords perhaps the best view of the

chromatin and its true spacial relations. Other stains and fixatives can be used with excellent results.

Except for the blepharoplasts, the most conspicuous structures of the spermatozoid are the four chromoneme threads. Following certain fixatives these structures appear to be diametrically uniform chromatic filaments which extend almost from end to end in the spermatozoid. Following other fixations, the chromatin filaments are seen as thin, granular threads, that is, as chromonemata. In the widest part of the spermatozoid body, the four chromonemata are almost invariably found to be in two pairs. At times, especially in the thinner parts of the spermatozoid, only two chromatic threads can be found, but these are often more conspicuous. Still further forward, only one chromatic thread can be seen on most occasions. Such observations would seem to indicate that four continuous chromonemata extend, with varying distinctness from each other, over a considerable length of the spermatozoid. They are always peripheral in position and in cross-section views of the spiralled body they are regularly found on the outer and posterior sides showing a tendency to lie in two pairs. They may be seen in osmium-aceto-carmine preparations, but their true spacial relations are less conspicuous by this technic. No discontinuity in the chromonemata has been observed.

Besides the four chromonemata in the karyolymph portion of the spermatozoid, on rare occasions there was seen one other much less conspicuous granular thread. This very delicate filament has been observed only on the blepharoplast side of the spermatozoid body and in one instance was clearly parted into two filaments for a distance of about 8 microns. Although it may be composed of chromatin, this thread is definitely not identical with the chromonemata which may be concurrently in evidence.

No other structural elements were seen in the karyolymph by any technic employed.

The cilia-bearing band. The two blepharoplasts rest in intimate contact with a distinct portion of the body which Yuasa (46) has referred to as the "cilia-bearing band." This band together with the blepharoplasts will be referred to as the *locomotor portion*.

In the case of the *Marsilea* spermatozoid, the cilia-bearing band is quite small and inconspicuous and, perhaps because of this, has been demonstrated by only two technics.

The stains successfully used by Mühldorf, Dracinschi, and Yuasa

have failed to discriminatingly stain this portion of the *Marsilea* spermatozoid. With iodine green, however, when this is used with osmium-treated material, a bi-chromatic staining is easily affected. When such preparations are studied with the most favorable type of heterochromatic yellow-green light, the blepharoplasts and the chromatin-bearing portion appear brownish-red and the remaining part of the spermatozoid body remains blue-green in color. This blue-green portion is the cilia-bearing band and varies in width from about one micron at its widest point to about .4 micron at the anterior end. In the posterior coils it may be seen from either one or both sides, whereas in the anterior coils it is constantly seen from both sides of the body.

This cilia-bearing band and its internal structure can be analysed more successfully by a steaming process which differentially removes various parts of the spermatozoid in a definite sequence according to their nature. A large drop of water containing many free-swimming spermatozooids is placed on a slide which is then put horizontally (right side up) over a beaker of steaming water. At necessary intervals hot water is added to the slide. After five to ten minutes of this treatment the water is allowed to evaporate completely. Sometime after the slide is dry on its upper side, the slide is cooled in water and stained with basic fuchsin. It is then dried quickly and mounted in balsam.

In one such preparation, it is usually possible to find fully extended spermatozooids in almost all stages of disorganization. Some are almost entirely normal in their constituent parts, that is, the blepharoplasts, cilia-bearing band, and the chromatin-bearing portion are all present without noteworthy structural change. Other spermatozooids show a first step in disintegration. This consists in the gradual loss of the karyolymph portion. The karyolymph breaks up into a number of small globules (Figure 20) which frequently can be seen clinging to the flagella. These globules of karyolymph substance move along the flagella away from the spermatozoid body and leave behind the locomotor portion of the spermatozoid. This is composed of the blepharoplasts, the cilia-bearing band, and the flagella (Figure 21). The next phase of disorganization occurs significantly later. This consists of a break-down of the cilia-bearing band. The ground substance of this band passes off in the form of small globules much as the karyolymph becomes removed (Figure 22). After this ground substance has disappeared, there remain two distinct types of structure. The first of these is the pair of blepharoplasts and the second is the flagellar apparatus (Figures 23, 24). At this phase of the break-down, the flagellar

apparatus is seen to consist of two strands of unstaining material. These strands may or may not continue to lie in a position parallel to the blepharoplasts. One of these strands gives rise to the flagella. This *ciliophore*, as it will be designated here, is of the same diameter and is composed of the same substance as the flagella. The second strand is almost identical with this functional ciliophore, but it bears no flagella or other outgrowths. On some occasions it becomes entangled with the functional ciliophore, this being due to the treatment. In the undisturbed spermatozoid these two ciliophores are parallel strands (Figure 19), the non-functional one being the innermost. The non-functional strand is presumably an undeveloped ciliophore, in view of the phylogenetic history of the flagellar apparatus and also in view of the flagellar relations in certain more advanced polypodiaceous spermatozoids where two, three, or four rows of flagella exist in contrast to one row in *Marsilea*.

As the disintegration progresses, the flagella lose their customary form and become globules of flagellar substance (Figure 24). When the disorganization proceeds further, the flagellar globules become larger and the flagella become shorter. The non-functional ciliophore concurrently shows evidence of decomposition over its entire length. The blepharoplasts are the last structures to survive the treatment. No evidence of a fibrillar composition of the flagella has been noted by any of various technics.

On the basis of this morphological analysis, the *Marsilea* spermatozoid consists essentially of the following parts (Figure 25):

- (1) The two blepharoplasts.
- (2) The cilia-bearing band which is composed of a "ground substance" and two ciliophores, one of which gives rise to the flagella.
- (3) The chromatin-bearing portion which is composed of karyolymph and four chromonemata.
- (4) The membrane which encloses the entire spermatozoid, exclusive of the flagella.

DISCUSSION

Spermatoteleosis

The cytoplasmic cap. In the literature on spermatogenesis in ferns we find no accurate description of the rôle of the cytoplasm during spermatoteleosis. It seems agreed generally that the cytoplasm of the spermatid becomes diminished in quantity as the metamorphosis progresses and that the cytoplasm is eventually found in the posterior

vesicle. In some instances it is believed that a portion of the cytoplasm contributes to the material of the spermatozoid. In *Marsilea* it is evident that the cytoplasm does not decrease to any noticeable extent during the metamorphosis of the spermatid. The portion of the cytoplasm which contributes to the material of the vesicle represents but a small part of the whole, since the vesicle consists largely of vacuoles and starch grains. The remainder of the cytoplasm resolves itself into the cytoplasmic cap and appears to represent all the remaining cytoplasm. •

The spiral cone of cytoplasm with its concavity in which the spermatozoid body lies during the earlier phases of its development was seen by Shaw (35) who also figured it (Plate XI, figure 20).

If we may judge by the figures for *Nephrodium* given by Yamanouchi (44), his figure 32 seems especially to suggest that he saw the cytoplasm on the outside of the spermatozoid spiral in this phase of spermatoteleosis. This seems to be borne out by the position in which he figures the "Nebenkern" for in *Marsilea* a body which compares favorably with his description of the "Nebenkern" is frequently found in this posterior region of the cytoplasmic cone during a corresponding phase of maturation. These and other features of Yamanouchi's description suggest that the *Nephrodium* type of development is essentially identical with that of *Marsilea*. It would therefore seem reasonable to suspect the presence of cytoplasmic caps in all of the polypodiaceous ferns. In *Marsilea* the "Nebenkern" substance is presumably chondriosomal in origin.

It is of particular value to recall at this time that Yuasa has described two "plasma-blocks" associated with the *Isoetes* spermatozoid (49, figure 6). One of these is in the usual posterior position whereas the other is found near the anterior end. This suggests that the posterior one is the vesicle and the anterior one is homologous to the cytoplasmic cap of *Marsilea*. A review of the literature will almost inevitably lead one to the suspicion that the ferns and fern allies probably all produce individual structures which are homologous to the vesicle of *Marsilea* and individual structures which are homologous to the cytoplasmic cap of this form. That homologies of this type may exist throughout the pteridophytes is no more remarkable than the homologies which are known to exist in other features of their spermatozooids or spermatogenous divisions.

The spermatozoid of *Equisetum* does not present an obstacle to this line of reasoning for the vacuolate posterior cytoplasm would be a vesicle

by homology. Even in *Chara* the spermatozoid is found to have a posterior, starch-bearing mass of cytoplasm (Mühldorf, 28). This portion, by homology, would also be a vesicle. It seems obvious that true vesicles are very general in the bryophytes. The presence of cytoplasmic caps in plants other than *Marsilea* (bryophytes, pteridophytes, and certain thallophytes), however probable their existence may seem, remains to be established by further investigations.

The elongating blepharoplasts. The ontogeny of the blepharoplast in the ferns has been studied and described in greater detail than any other single feature of spermatogenesis.

Investigators seem to be of the opinion that the blepharoplast elongates outside the nuclear membrane (44, 1, 32, 33, 30, 50). In some instances the blepharoplast is in close union with the nuclear membrane from the earliest period of elongation (*Nephrodium*, 44; *Marsilea*, 33; *Polypodium* 30). In other instances the blepharoplast undergoes some elongation before it becomes applied to the membrane (*Equisetum*, 32; *Notogramme* and *Pteris*, 50; *Salvinia*, 45). In some forms the blepharoplast is said to develop noticeably in advance of the nuclear membrane (*Marsilea*, 33; *Polypodium*, 30). Weier (41) working with mosses and Miss Rankin (30) working with *Polypodium* have agreed that the blepharoplasts are outside the nuclear membrane during the period of elongation. This is especially significant because both investigators employed cytoplasmic fixatives and technics which should be particularly favorable for the study of this feature. Weier further points out that in *Polytrichum* the two blepharoplasts eventually separate from the nuclear portion of the spermatozoid body and become cilia. It would seem, therefore, that this detail has been disposed of satisfactorily.

The author's observations, when considered entirely apart from the findings of others, would definitely support the interpretation that the blepharoplasts of *Marsilea* are within the nuclear membrane during the greater part of their elongation. During the earliest phases of blepharoplast growth these structures are seen as two rigid and thick bodies within the undeformed spermatid nucleus. As these blepharoplasts grow in length they protrude into the nuclear membrane very much as a pencil would distort an inflated rubber balloon from the inside. By the time one full spiral has developed, the blepharoplasts are closely invested with this difficultly-discernible membrane. When the blepharoplasts are preferentially dissolved from the spermatozoid, it is found, by studying various stages of dissolution, that the ciliophores remain undisturbed within the gamete membrane and in favorable material the membrane can be seen to encompass the partially digested blepharo-

plasts. It would further seem entirely likely that all spermatozoid components are contained within a protective membrane. For these various reasons it is suggested that the *Marsilea* blepharoplasts are intranuclear structures during their elongation and at maturity.

The blepharoplasts in all phases of their existence are distinctly rigid bodies. Spermatozooids fixed with aceto-carmin which swells the component substances and spermatozooids fixed with cytoplasmic fluids strong in formalin which shrinks these components strongly indicate such a rigidity of the blepharoplasts. Hot water causes the spermatozooids to unroll; ostensibly this is due to the straightening of the blepharoplasts. When the blepharoplasts are preferentially dissolved from a spermatozoid, this body either loses its spiral form completely or simply collapses into a flaccid, flattened mass. It would seem reasonably justifiable, then, to regard the blepharoplasts as the form-giving elements of the spermatozooids.

These blepharoplast conditions in *Marsilea*, if they exist in fact, do not necessarily conflict with other interpretations on cytologically more primitive forms. An effort will be made elsewhere to indicate that intranuclear blepharoplasts could be interpreted as phylogenetically advanced types.

The Mature Spermatozoid

The major structural features of the spermatozoid as described for *Marsilea* have been reported previously in other plants.

Weier (41) has reported that the blepharoplast of *Polytrichum* is a double structure and has referred to these as two blepharoplasts. In the algae, *Oedogonium* (22) and *Derbesia* (13) are known to form double blepharoplasts comparable to those of *Marsilea* (see also Fritsch, 17). The doubleness of the blepharoplast as reported for *Marsilea* by Sharp (33), if we consider his figure 42, is not the same condition referred to above. In his drawing, the lower strand is almost certainly identical with the two ciliophores described in this paper (compare with figure 19). This interpretation would seem to explain why most investigators have believed the blepharoplasts of the ferns to be cilia-bearing organs.

The existence of two blepharoplasts of the *Marsilea* type in other spermatozooids of the *Pteris* type and including the spermatozoid of *Equisetum* would seem highly probable. A more detailed discussion of double blepharoplasts in algae, fungi, bryophytes, and ferns will be presented in a later paper and an effort will be made to indicate the probable homology and evolutionary history of these structures.

The structural organization of the chromatin in fern spermatozooids

has never been accurately described. Most investigators have believed with Yamanouchi that the chromatin becomes greatly compacted during spermatoteleosis as the spermatid nucleus becomes reduced in volume. Eventually the chromatin is said to become homogeneous and unstaining. Most investigators have generally agreed that the entire spermatozoid consists of a nuclear portion and a blepharoplast from which the cilia originate. Although more recent investigations have shown that the flagella certainly do not arise from the blepharoplast, it is still generally thought that the chromatin of the spermatozoid is greatly compacted, homogeneous, and unstaining or that the chromatin exists as a fairly coarse coil of material in the nuclear portion of the body. We might analyse these interpretations more closely in relation to the conditions found in *Marsilea*.

It seems generally agreed that the spermatid nucleus becomes greatly reduced in volume during its metamorphosis. By direct observation alone, this would seem to be the case. If, however, we make measurements and compute the volume of the spermatid nucleus before spermatoteleosis and compare this volume with the volume of the karyolymph portion of the mature spermatozoid, we find that the reduction in volume is more apparent than real. With critical technics, such measurements can be accurately made. The volume of the spermatid nuclei, before transformations in form become initiated, is approximately $110 \mu^3$. The volume of the chromatin-bearing portion of the mature spermatozoid is approximately $100 \mu^3$. If the measurements are accurate to within 10%, which they seem to be, we can see that a contraction of the nuclear portion of the spermatid is less an actual one than a casual observation would lead us to believe. The interpretation that the chromatin becomes greatly compacted in the spermatozoid seems therefore to be an untenable one.

The chromatin in the *Marsilea* spermatozoid exists as four parallel chromonemata which extend in a linear fashion from the posterior end of the spermatozoid well forward into the anterior end. These chromonemata do not form a spring-like coil within the body as described for *Leptogramme* by Yuasa (47). It is true that many spermatozooids appear to have such a nuclear structure as Yuasa describes. The author at first believed such a condition to exist in *Marsilea* (see figures 10, 19). This interpretation was based on preparations of the type studied by Yuasa. In every such case, the "internal spiral" was found to be an optical artifact caused by the flagella which become closely and firmly wound about the spermatozoid body. That no internal coil of chroma-

tin materials exists may be conveniently demonstrated during the first five minutes of staining in osmium-aceto-carmin preparations.

In support of the present analysis of chromonemata in *Marsilea*, we will recall that Johnson (21) has described the chromatin of *Plagiochila*, a liverwort, to be in the form of chromoneme threads. The author believes it reasonable, therefore, to suggest that a similar disposition of chromatin may be found to exist in the spermatozoids of other ferns, in bryophytes, and perhaps even in the *Characeae*. Certainly this detail would seem to merit attention in further work on these forms, especially since it is difficult to understand the presence of four chromonemata in the spermatozoid of *Marsilea*, a fern whose haploid chromosome number is eight.

The cilia-bearing band of the fern spermatozoid was first reported by Mühldorf (27). Since that time, others have found similar cilia-bearing bands in other fern spermatozoids (Dracinschi, 14, 15, 16; Yuasa, 46, 47, 48, 50, 51). In order to make fully clear the confusion which this discovery has caused, it seems desirable to trace briefly the history of another type of cilia-bearing band as it occurs in other spermatozoids.

Webber, in October 1897 (39), applied the term "blepharoplast" to the structure in the *Zamia* spermatozoid which gives rise to the cilia. This is often referred to as a cilia-bearing band and is characteristically a deeply-staining spiral band. The term "blepharoplast" by derivation means a cilia-forming organ. In the case of *Zamia* as in other gymnosperms, the blepharoplasts are strictly cilia-forming organs since the cilia seem to sprout directly from the chromatic band which bears this name. Almost concurrently with Webber's discovery of the *Zamia* blepharoplast, Belajeff (5) announced the presence of a similar spiral organ in various ferns. He thought this band gives rise to the flagella and referred to it as a cilia-bearing band or "Nebenkern." In the following years it became generally accepted that the chromatic, spiral bands of the fern spermatozoids are homologous to the blepharoplasts of the gymnosperms. The cilia of the ferns were said to originate from the fern blepharoplasts and this cilia-bearing function was believed to be general for all blepharoplasts. This interpretation was widely accepted until Mühldorf in 1930 pointed out that the cilia of certain fern spermatozoids do not originate from this blepharoplast, but that the cilia are outgrowths of a special band which lies in contact with the blepharoplast.

Miss Dracinschi, working in the same laboratory with Mühldorf,

investigated the morphology of a number of fern spermatozoids (14, 15, 16). In 1930 she reported on *Nephrodium* and various other forms. In this publication she criticized Shaw (35) for his use of the term "blepharoplast" in connection with the ferns (*Marsilea* and *Onoclea*) because, she states, this designation positively ill-fits the structure in question since the flagella are not attached to it. She therefore referred to the blepharoplast as a "Randsaum." At the same time she reported the existence of a basal granule ("Basalkorn") at the base of each flagellum. She also attempted to analyse the nature of the "Randsaum" and reported that silver and osmium impregnation methods give negative (?) results with this organ. She summarized this analysis as follows: "Da am vollständig reifen Spermium Mitochondrien nicht zu finden sind, so ist es nicht ausgeschlossen, dass dieser Randsaum Mitochondrien körper sein könnte." A stiff, basal sheath or "Stiel" was also reported to be present on every flagellum.

In a later publication (16) Miss Dracinschi again pointed out that the "Randsaum" is chondriosomal in nature. She states here (page 96), "Dieser 'Randsaum' wurde von Belajeff fälschlich als 'cilienbildener Faden' (Blepharoplast) bezeichnet, da er glaubte, dass von diesem Gebilde die Geißeln ihren Ursprung nähmen und daran befestigt seien. Auf Grund früherer Untersuchungen (Dracinschi) gelang es für die *Filicales* Spermien zu beweisen, dass dieser Randsaum ein Chondriosom ist, da er auf spezielle Chondriosomefärbungen positive reagiert. Der Randsaum von *Equisetum* verhält sich genau wie bei den *Filicales* Spermien und ist daher auch als ein Chondriosom anzusehen. Der Name 'Blepharoplast' kommt ihm also nicht mehr zu, . . ."

The results of her investigations may be briefly summarized as follows: (1) the term "blepharoplast" is not appropriate to this structure, (2) the "Randsaum" is to be regarded as a chondriosome, (3) each flagellum bears at its insertion point a basal granule or "Basalkorn," and (4) the locomotor portion or "Stamm" is of "solider plasmatischer Beschaffenheit" and except for the "Basalkörner" and the "Randsaum" has no other structure. These points will be discussed in detail, since they are of great importance if Miss Dracinschi is correct, but first it seems desirable to review the results of Yuasa's investigations.

Yuasa agrees with Miss Dracinschi in several notable points, but he uses a different terminology. The "randsaum" (blepharoplast) he refers to as the "border-brim." The "Stamm" he refers to as a cytoplasmic "cilia-bearing band." It should be noted that he uses the expression "cilia-bearing band" in a different sense than it was used by

Webber (40) and Chamberlain (12). In 1933, Yuasa (47) suggested, "that the so-called blepharoplast which appears during spermatogenesis probably corresponds to the part that includes both the cilia-bearing band and border-brim." In 1934 he described (50) the ontogenetic history of the blepharoplast and the cilia-bearing band as follows: "One edge of the blepharoplast stains deeply and develops into the border-brim, the remaining portion of the blepharoplast coalesces with the nucleus, but the unstained or faintly stained portion remains between the border-brim and the nucleus. This is the cilia-bearing band, . . ."

From these investigations certain facts seem quite clear and are supported by my observations on *Marsilea*. The polypodiaceous type of spermatozoid, exclusive of the vesicle, consists of (1) two blepharoplasts ("Randsaum," "border-brim"), (2) a "cilia-bearing band" ("Stamm"), and (3) a chromatin-bearing portion ("nucleus"). The presence of a basal sheath is also verified, but in living material this is quite flexible. In fixed material, however, the sheath may be very stiff.

Certain observation made by Miss Dracinschi have not been verified. She finds a basal granule at the point of flagellar insertion. Yuasa mentions a "basal swollen point." I have used the technics outlined by Miss Dracinschi to demonstrate these granules but find none. Very often one may see distinctly darkened areas where she finds these granules, but such granule-like features in most instances are almost certainly optical artifacts however excellent the optical system may be. The existence of a functional ciliophore in *Marsilea* as revealed by a process of steaming would seem to provide an adequate denial to the reported occurrence of basal granules.

Miss Drachinschi also states that the term "blepharoplast" is not appropriate to the structure which she renames the "Randsaum" and which Yuasa renames the "border-brim." It would seem, however, that the principle of homology should not be ignored in this matter. It is indubitably clear that the blepharoplasts of the ferns are homologues of the gymnosperm and bryophyte blepharoplasts (Sharp, 32, 33, 34). That the fern blepharoplasts are not directly functional in developing flagella is a condition which does not determine homology. The term "blepharoplast" remains as thoroughly appropriate to those structures in ferns so designated by Sharp as it is when applied to the cilia-bearing structure of the gymnosperm spermatozoid. It would seem, then, that the terms "Randsaum" and "border-brim" may be

regarded as alternate names for an organ which is most appropriately named "blepharoplast." Yuasa has more recently suggested that the cilia-bearing part together with the border-brim might be interpreted as the blepharoplast. Although this suggestion seems thoroughly sound, it introduces at once several new difficulties in terminology. The nature of these difficulties will become more apparent in the following pages.

Another interpretation made by Miss Dracinschi and which the present investigation has failed to substantiate concerns the "cilia-bearing band." Mühldorf (28), Dracinschi (14, 15, 16), Yuasa (47) and others have agreed that the cilia-bearing band is cytoplasmic in nature. They base their interpretations largely upon preparations of the osmium-treated type although other evidence is brought forward. The basis for this identification would seem to be somewhat inadequate. If the component substance of the cilia-bearing band be cytoplasmic in character, then we shall expect ordinarily to obtain certain characteristic differences between this substance and the karyolymph when we use fixing fluids of the usual type, such as those of Nawaschin, Lewitsky, and Benda. But none of these fixations discloses the presence of two such types of substances in the spermatozoid body regardless of the stain or stains used. Yet these fixatives set the cytoplasm in strong contrast with the karyolymph in all spermatogenous cells even in unstained material. Aceto-carmin preparations disclose an equally prominent contrast of these substances. With ordinary angiospermous material this contrast between cytoplasm and nuclear lymph is usually much less pronounced or is distinctly absent. In the spermatogenous cell of *Marsilea*, however, it is easily apparent that the cytoplasm is discolored by the fixing fluid or subsequently stains heavily (Figure 14). These facts would seem to deny the presence of ordinary cytoplasm in the mature spermatozoid. That osmium-treated material affords a differential staining with acid and basic dyes is insufficient evidence that if one component is nuclear substance that the other must be cytoplasmic material. Likewise, it does not necessarily follow from such differential staining that if the cilia-bearing band of the fern spermatozoid stains in the same manner as the anterior end of the bryophyte spermatozoid that the two portions are necessarily composed of the same material.

Yuasa (50) has reported that the blepharoplast itself gives rise to the cilia-bearing band. This development has not been observed during the present investigation but various lines of evidence support Yuasa's findings in this matter. However, he assumes that the cilia-bearing

band is therefore cytoplasmic substance. Since the author believes that the blepharoplasts are contained within the nuclear membrane at the time that this cilia-bearing band becomes organized into its final form he would interpret Yuasa's observations to indicate that this band originates within the nuclear membrane. This would make it seem less likely that the cilia-bearing band is composed of cytoplasm. The author's analysis on the nature of the blepharoplasts and the cilia-bearing band has been based on an entirely different line of evidence and is presented in part in the next section.

The Blepharoplasts and the Cilia-Bearing Band

The fundamental nature of the blepharoplast has remained a perplexing problem for almost forty years. The earlier investigators were unable to come into agreement in this matter, largely because of conflicting information which arose out of the inadequate microtechnical and microchemical information of their time. In recent years the literature has become greatly enriched in many respects and we are now able to analyse more exactly many of the problems which at first were highly debatable. This condition applies particularly well to the problem of blepharoplasts and centrosomes.

It seems clear today that the blepharoplasts of the thallophytes, bryophytes, pteridophytes, and gymnosperms are all strictly homologous structures. It would likewise appear obvious that blepharoplasts, in many instances, are ontogenetically derived from centrosomal material. These deductions, however, tell us nothing of the real nature of the blepharoplast. In an attempt to solve this problem, various suggestions have been made.

One suggestion has been more widely accepted than any other. Most investigators have agreed with Webber (40) that the blepharoplast, as a centrosome-like body, is an organ *sui generis* and that it appears and develops in the cytoplasm during the spermatogenous divisions. These investigators hold the view or imply that blepharoplasts are cytoplasmic organs.

Another suggestion found in the literature is that the blepharoplast represents the chondriosomal material of the spermatid (Motte, 26; Dracinschi, 14, 15, 16).

Certain others believe to have adduced evidence that the blepharoplast substance finds its ontogenetic origin within the nucleus (20, 24, 12, 13, 42, 4, 11, and others) and most of these investigators hold to the view that the blepharoplast finds its origin specifically in the nucleolus.

The "Hautschicht" origin which Strasburger proposed has also received frequent comment, but it is doubtful that Strasburger would maintain this view today.

We find therefore, that three outstanding points of view find support in the cytological literature at the present time: (1) the blepharoplast is a cytoplasmic organ *sui generis*, (2) the blepharoplast represents the spermatid chondriome, and (3) the blepharoplast is a nucleolar derivative.

None of these three views has been shown to satisfy all the events of spermatogenesis and none of them has been shown to provide us with a better understanding of the various phenomena which are either ontogenetically or phylogenetically associated with the development of the blepharoplasts. The author believes that we can at the present time establish the true nature of the blepharoplasts and formulate satisfactory answers to the many questions which have been raised in this connection. To accomplish this it appears desirable to inspect the suggestions which have been made concerning the nature of the blepharoplast.

The blepharoplast as an organ sui generis: If we agree that centrosomal blepharoplasts are cytoplasmic structures which originate *de novo* in the cytoplasm, then our search is at an end. This interpretation, however, affords us no explanation of the questions which have been raised in regard to this structure.

The blepharoplast as chondriosomal material: Motte (26) was the first to present a serious analysis in favor of this view, but Miss Dracinschi (14, 16, 17) has subsequently advanced evidence in favor of the same interpretation.

Both investigators believe the blepharoplast substance to be chondriosomal material because it gives a positive reaction to specific chondriosomal technics. It is quite true that the blepharoplasts do stain more intensely following chondriosomal fixations than they do following the "nuclear type" of fixation. It is likewise true that the blepharoplasts of living material stain quite distinctly with Janus green. But we cannot ignore the fact that the blepharoplasts also stain heavily following the usual nuclear fixatives. They stain less heavily with aceto-carmine, but their component substance persists indefinitely even in this quantity of acetic acid without a noteworthy change in form. This behavior is certainly not characteristic of ordinary chondriome substance. Can we, then, be justified in calling this blepharoplast structure a chondriome?

The blepharoplast as a derivative of the nucleolus: That the blepharoplast is derived from the nucleolus has been advocated by various investigators. This interpretation has been seriously criticized in many ways. At the present time the evidence in favor of this interpretation can be classified as being of two types; that evidence which concerns the mature blepharoplasts alone, and that evidence which is available from studies on the ontogenetic and phylogenetic history of the blepharoplasts. Only part of the first of these two types of evidence will be discussed here. Other evidence will be presented in a later paper.

In an earlier section the suggestion was made that the *Marsilea* blepharoplasts are probably mechanically responsible for the spiral form of the spermatozoid. If this be true, we should expect to find the spermatozoid body rigidly spiralled as long as these structures are present and the body rigidity should disappear when the blepharoplasts are selectively removed from the spermatozoid. The question arises, then, as to what solvent will remove these structures without materially affecting the remainder of the body. Since we are interested in determining a relation between blepharoplasts and nucleoli, or the absence of such a relation, we would naturally look for technics which are specific to nucleoli. For this purpose, staining reactions may be of doubtful value, but various differential solubility tests appear to be more reliable.

Two types of "specific" nucleolar technics are available. With the first of these we may remove essentially all material from a meristematic type of protoplast except the nucleolar substance. This may be accomplished by treating living material with hot water (60 to 90°C. for 5 to 30 minutes). By the second type of technic we can selectively remove the nucleolus (43). Only three specific solvents for nucleoli are known (HCl, sodium borate, and sodium formiate) (43). This would strongly suggest that the nucleoli are extremely individualistic structures in their chemical composition and enhances the value of such tests.

If we fix a root-tip (*Allium*, *Vicia*, etc.) with hot water and stain sections with hematoxylin, we find that the cell contents are removed in a very characteristic sequence. Two substances ordinarily remain in such preparations. The first of these is entirely unstained and consists of the "kinoplasmic" substances of the cell. These are the plasma and nuclear membranes and the strands of similar material which connect these membranes with each other and the strands which connect the nuclear membrane with the nucleolus. The second substance which remains in these preparations stains very intensely and consists of the

(3) The blepharoplasts are composed of a substance which in the higher plant nucleolus has been referred to as "nucleolin."

(4) The cilia-bearing band of the spermatozoid is composed largely of the same substance which in the higher plant nucleolus has been referred to as "plastin."

(5) The chromatin exists in the form of four linear chromonemata.

(6) The karyolymph of the spermatid nucleus is present in the fully mature spermatozoid without an appreciable decrease in quantity.

(7) The cilia are kinoplasmic structures that originate from one of two ciliophores located within the nucleolar material of the spermatozoid. There are no basal granules in the *Marsilea* spermatozoid.

The evidence in favor of nucleolar blepharoplasts is by no means exhausted with this type of analysis. We might recall in favor of this interpretation that only one normal cell constituent, the nucleolus, has the property of fragmenting into bead-like chains and spirals similar to those of growing blepharoplasts. This nucleolar feature has been described and illustrated by Zirkle (52) (Plates 5 and 6, Figures 25, 26, 33a, b, 34a, b). Only one normal cell constituent, the nucleolus, is a two-component structure whose components react toward ordinary fixatives and stains as do the blepharoplasts and the cilia-bearing band of the mature spermatozoid.

That the entire spermatozoid consists of essentially nuclear material is also indicated by the report on fertilization in *Nephrodium* made by Yamanouchi (44) who observed an entire spermatozoid, including the flagella, within the egg nucleus during fertilization.

An effort will be made in a later paper to trace the probable evolutionary history of the blepharoplast and to bring forward other lines of evidence which support the notion of nucleolar blepharoplasts independently of the facts given here.

From the foregoing account, however, it would seem highly probable that the spermatozoid of *Marsilea* consists essentially of nuclear material, the predominate constituent substances being five distinguishable kinds: chromatin, karyolymph, kinoplasm, nucleolin, and plastin.

SUMMARY

(1) A study of spermatogenesis in *Marsilea* has been made and a detailed analysis of the mature spermatozoid is presented. The major events of spermatoteleosis are also described.

(2) During spermatoteleosis, most of the spermatid cytoplasm be-

comes reorganized into a cytoplasmic cap without an appreciable decrease in volume. This cap separates from the spermatozoid during antheridial dehiscence. The blepharoplasts elongate within the spermatid nucleus and are responsible for the final form of the spermatozoid. The karyolymph of the spermatid nucleus does not become appreciably reduced in volume during spermatoteleosis.

(3) The mature spermatozoid consists of at least five types of structurally organized substances: chromatin, karyolymph, kinoplasm, nucleolin, and plastin. Since these materials are characteristically found within the nucleus, we may regard the mature spermatozoid as being entirely nuclear in composition. The swimming spermatozoid, except for its vesicle, being devoid of cytoplasm and bearing a complete complement of nuclear materials, is then, a motile nucleus.

(4) The chromatin exists as four linear, parallel, and peripherally placed chromonemata which extend throughout the length of the spermatozoid.

(5) Most of the spermatozoid body consists of homogeneous and unstaining karyolymph.

(6) The flagella are attached to the spermatozoid body on its forward side and arise from one of two parallel strands of flagellar material located within the cilia-bearing band. These two ciliophores extend over the entire length of the spermatozoid. The substance of the flagellar apparatus is kinoplasm and there are no basal granules in the spermatozoid.

(7) A cilia-bearing band rests in close association with the blepharoplasts and consists of plastin which is a prominent component of ordinary nucleoli.

(8) The blepharoplast structure is not a single, chromatic rod of substance, but is composed of two identical and parallel strands of material. The blepharoplast substance is similar to nucleolin of ordinary intranuclear nucleoli.

(9) The spermatozoid structures, exclusive of the flagella, are invested by a single membrane, this being the membrane of the former spermatid nucleus.

It is a pleasure to acknowledge the valuable assistance given the author by Dr. John N. Couch and Dr. W. C. Coker. The writer is particularly indebted to Dr. B. W. Wells who has given much valuable advice throughout this investigation and who made the drawing shown on Plate 35. Dr. Lester W. Sharp of Cornell University, who suggested

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DESCRIPTION OF PLATES

All photomicrographs were made with a Spencer microscope equipped with a 1.25 N. A. Abbe condenser and a 1.8 mm., N. A. 1.25, achromatic objective in combination with a Leitz "Periplan" ocular (12X), using full numerical aperture.

PLATE 31

- Fig. 1. Spermatozoid stained with carbol fuchsin and showing "end pieces" on the flagella. $\times 1000$.
 Fig. 2. Partially extended spermatozoid. Stained with carbol fuchsin. $\times 1250$.
 Fig. 3. A swimming spermatozoid deprived of its vesicle. Stained with safranin O. $\times 1250$.
 Fig. 4. An extended spiral almost detached from the vesicle. Carbol fuchsin. $\times 1000$.
 Fig. 5. Two swimming spermatozooids as seen shortly before fertilization. The large, hyaline, secondary vesicles do not show. Fixed with osmium fumes and stained with safranin O. $\times 2300$.
 Fig. 6. Swimming spermatozooids like those of figure 5. Photographed to show the coiled blepharoplasts. Safranin O. $\times 1250$.
 Fig. 7. A group of about twenty spermatozooids like those of figure 6 after treatment with HCl. Stained with basic fuchsin. $\times 1375$.

PLATE 32

- Fig. 8. Spermatozoid fixed with osmium fumes and stained with aceto-carmin. *a*, blepharoplasts. *b*, chromonemata. $\times 2300$.
 Fig. 9. Spermatozoid; osmic fixation and aceto-carmin staining. *a*, blepharoplasts. *b*, chromonemata. $\times 2300$.
 Fig. 10. Spermatozoid; osmium fumes and safranin O. *a*, blepharoplasts. *b*, ciliophores. *c*, flagella wound about body. $\times 1250$.
 Fig. 11. Spermatozoid fixed with osmium fumes and stained with neutral red. Flagella seen in negative view. $\times 2100$.
 Fig. 12. Spermatozoid treated with HCl; basic fuchsin. The blepharoplasts and the cilia-bearing band are removed. The chromonemata and the ciliophores remain in the body. $\times 1500$.
 Fig. 13. Spermatozooids fixed with osmium fumes; iodine green. *a*, blepharoplasts. *b*, cilia-bearing band. $\times 2000$.
 Fig. 14. Sub-median section through a microspore about 15 minutes before dehiscence. *a*, microspore wall. *b*, cytoplasmic cap. *c*, vesicle. *d*, blepharoplasts. Regaud's fixative and Heidenhain's hematoxylin. $\times 1150$.
 Fig. 15. Spermatozooids at the time of dehiscence. *a*, cytoplasmic cap. *b*, blepharoplasts. *c*, "Nebenkern." Regaud's fixative and hematoxylin. $\times 1150$.
 Fig. 16. Spermatozoid shortly before dehiscence. *a*, cytoplasmic cap. *b*, blepharoplasts. *c*, "Nebenkern." *d*, vesicle. Regaud's hematoxylin technic. $\times 2225$.

PLATE 33

- Fig. 17. Spermatozoid partly separated from the vesicle. *a*, vesicle. *b*, spermatozoid body. *c*, "end pieces" of the flagella. Osmium fumes and carbol fuchsin. $\times 2300$.
- Fig. 18. Spermatozoid after losing its vesicle. Osmium fumes and basic fuchsin. $\times 2000$.
- Fig. 19. Spermatozoid. Compare with figure 10 which has a greater depth of focus. *a*, blepharoplasts. *b*, ciliophores. *c*, flagella wound about body. Osmium fumes and safranin O. $\times 2000$.

PLATE 34

- Fig. 20. Spermatozoid treated with hot water. Karyolymph being removed in the form of small globules. Basic fuchsin. $\times 1375$.
- Fig. 21. Spermatozoid after the karyolymph is entirely removed. The body consists of blepharoplasts and the cilia-bearing band from which the flagella protrude. Basic fuchsin following hot water treatment. $\times 2200$.
- Fig. 22. Spermatozoid treated with hot water showing a slightly more advanced stage of break-down. The ground substance of the cilia-bearing band passing off as small globules. Basic fuchsin. $\times 1525$.
- Fig. 23. Spermatozoid treated with hot water, showing the remaining substance following the removal of the ground substance of the cilia-bearing band. $\times 1375$.
- Fig. 24. A late stage of break-down of a spermatozoid. *a*, blepharoplasts. *b*, functional ciliophore. *c*, partially dissolved flagellum. *d*, non-functional ciliophore, partly decomposed. Hot water treatment and basic fuchsin. $\times 1375$.

PLATE 35

- Fig. 25. A portion of a spermatozoid showing the major constituents. The two blepharoplasts are at the top. In the forward half of the spermatozoid, these may have occasional hyaline segments as shown. The ground substance of the cilia-bearing band lies in contact with the blepharoplasts and contains the two ciliophores. One of these bears flagella over the entire length of the spermatozoid. Four chromonemata are peripherally located in the karyolymph portion. The membrane of the former spermatid nucleus encloses all structures and forms the basal sheaths of the flagella.

PLATE 31

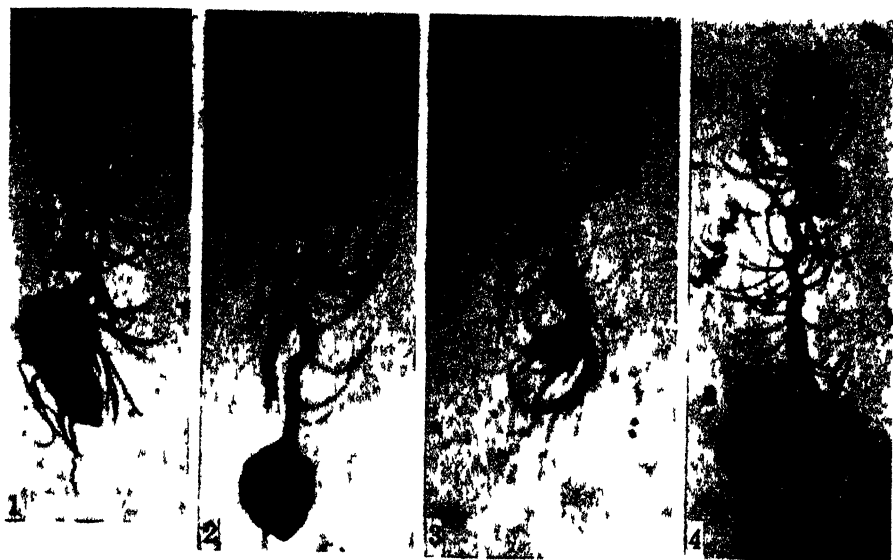


PLATE 32

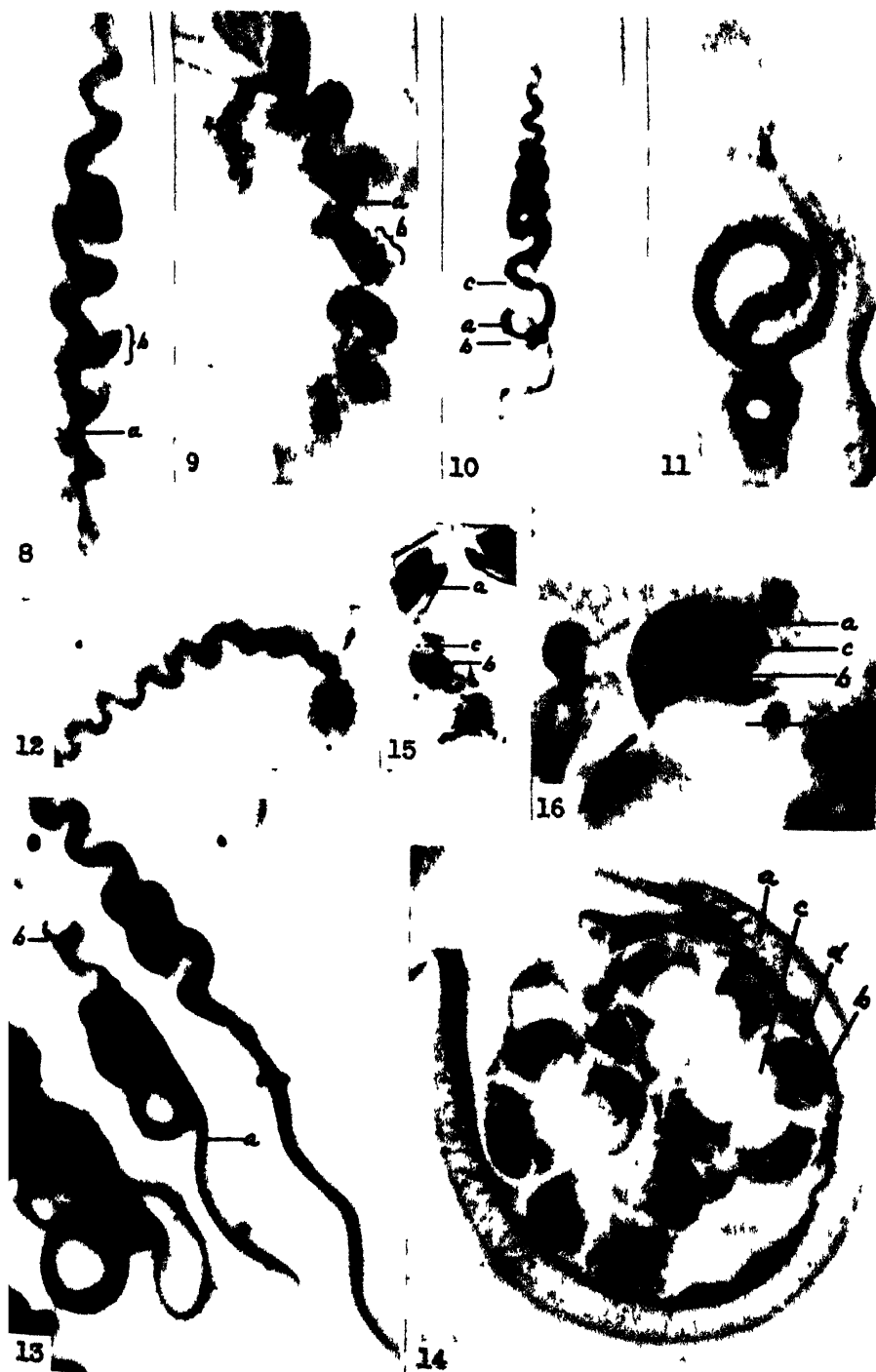
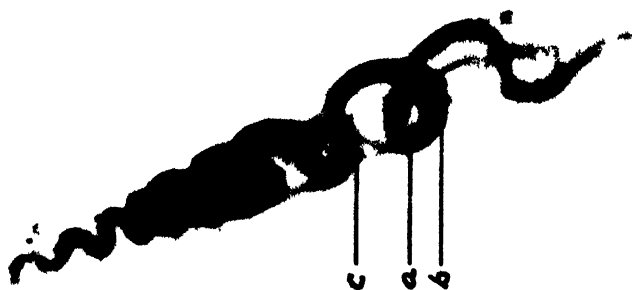


PLATE 33



19



21

PLATE 34

